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EEG AUDIOMETRY IN CHILDREN UNDER THREE YEARS OF AGE

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Abstract EEG-evoked response audiometry was performed on 241 children under 3 years of age One hundred were patients referred for evaluation of hearing and 141 were normal children A total of 498 recordings were made usually during sleep Many children in the patient group had severe mental or neurological impairments which made assessment by clinical audiometry difficult Evoked response audiometry often provided more complete diagnostic information earlier than could be obtained by other methods In some normal infants especially under 6 months of age evoked response thresholds during sleep were elevated It was possible to improve measurements near threshold by modifying recording techniques We conclude that EEG-evoked response audiometry is a valuable means of assessing the auditory capacity of the young or handicapped patient

The purpose of this paper is to present a summary of 4 years experience with computer averaged evoked response audiometry in the diagnosis of hearing loss in young children Recordings from the first 100 children under 3 years of age who were referred for evaluation of hearing and from 141 normal hearing children of the same age form the basis of this report Serial recordings were performed in some of the patients 140 in all In the normal group 358 recordings were performed before 3 years of age

EEG evoked response audiometry is predicated on the fact that suprathreshold auditory stimuli presented during brainwave recording cause alterations in ongoing brainwave patterns Recognition of sound induced EEG changes is often difficult because of the complexities of the background EEG In recent years the use of electronic signal averaging devices has greatly facilitated the development of evoked response audiometry as a reliable tool in audiology The application of averaging techniques depends on the fact that the brain response occurs at a fairly constant time after the stimulus and exhibits a relatively constant series of waves When a number of identical stimuli are presented and the EEG voltages occurring after each stimulus are averaged the time locked evoked response waves emerge as the variance of the averaged background EEG decreases

Evoked response audiometry now is being used in diagnostic audiology in several laboratories and the reliability of the method is well established in older children and adults (Davis et al 1967) However the most important potential clinical application of evoked response methods is in the testing of children whose age or mental or motor handicaps make voluntary audiometry uncertain or impossible This report adds information as to the validity and reliability of the technique in infants and it suggests some of the clinical situations where its application may be most useful (See also Rapin & Graziani 1967 Rapin & Bergman 1969 McCandless 1967 Suzuki guchi 1968 Engel & Y 1969) T some suggestions on the our

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Table 1

Diagnostic category	Number of subjects by age (months) at first EEG audiogram					
	0–6	6–12	12–18	18–24	24–36	Total
Congenital rubella syndrome	1	1	[illegible]	5	1	41
Congenital anomalies	11	5	1	0	0	1[illegible]
Kernicterus	2	1	[illegible]	1	1	9
Neonatal hyperbilirubinemia	1	[illegible]	1	0	1	[illegible]
Developmental retardation	3	1		1	4	13
Hearing loss	0	1	0	1	1	3
[illegible] or developmental [illegible]	3	0	1	0	0	4
Other	2	1	0	0	2	5
Total	3[illegible]	[illegible]	14	[illegible]	14	103

[illegible] head and neck [illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

ence as to instrumentation and methodology. Since the patients studied were young and in many cases severely handicapped, threshold measurements were sometimes extremely difficult. The method of comparison used in this study was based on the best estimate of sensitivity capacity by EEG and clinical techniques arrived at independently. When a reliable pure tone voluntary audiogram could be performed (usually when the child was about 3 years old), it was compared with clinical and EEG evaluations performed when the child was younger.

SUBJECTS

Patients

A brief summary of the clinical or diagnostic categories of the patients evaluated is given in Table 1.

During the rubella epidemic of 1964 referrals for audiometric study of all patients who were considered to be at risk as to rubella syndrome because of maternal history or viral and serological evidence of infection were sought. Some of these patients have been previously reported (Barnet & Lodge 1966, 1967). The other patients were evaluated mainly because the referring physician suspected a hearing loss or the diagnosis, e.g. kernicterus, was sometimes associated with hearing loss. A small group consisted of cases in which the referring physician wished to rule out specific sensory defect in patients who had generalized mental or motor retardation or cerebral palsy. There were several infants referred because they were being evaluated for adoption.

Normal subjects

The sources of this population were children living at two children's homes, well baby clinics, and children of hospital personnel. Some are being given serial evoked potential and developmental tests to follow the normal evolution of these measures of CNS function. All were full term babies with family histories negative for early hearing loss. They were products of normal pregnancy and birth and showed normal development as measured by pediatric and developmental test examinations.

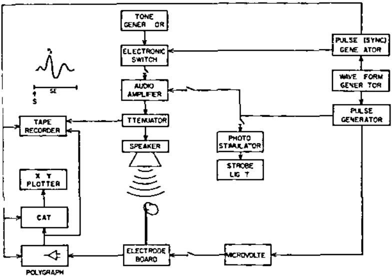


Fig 1 Simplified block diagram of experimental apparatus A typical auditory evoked response (*AER*) is also shown An upward deflection denotes positivity of the active electrode with respect to the reference The stimulus occurred at the beginning of the trace

Brief ENT and hearing evaluations which were given as part of the other examinations were within normal limits

METHODS

Almost all the tests were done while the children slept In infants under 9 months tests were performed without sedation at the baby s regular nap time Older patients were given chloral hydrate syrup p o (50 mg/kg) to induce sleep but it was always important for the technician to play a few minutes with the child and to take care that the child was comfortable and well fed None of the normal group were sedated About 5–10 of the children in this age group (normal and patient) had to be re scheduled because they were too active to permit a test session long enough to obtain all the information desired There was no child however on whom at least a partial test was not obtained

Scalp electrodes were applied at the vertex (C in the International Electrode System) and to the left and right of the vertex along the interaural line (C_3 & C_4) and in the mid occipital area (O) Interconnected mastoids or ear lobes were used as reference electrodes An electrode on the forehead was used as ground In some instances other scalp electrode placements and combinations were used as well as the electrooculogram and chin electromyogram Brainwaves were amplified using standard Tektronix or Grass amplifiers with a frequency band pass of 0 8 Hz – 250 Hz In some later recordings frequencies above 15 cycles were filtered out A paper recording of the ongoing EEG was obtained a well as the averaged evoked responses The EEG tracing was used to determine sleep patterns and to evaluate the technical quality of the recording Fig 1 shows a simplified diagram of the apparatus used The technician made a judgement on line as to whether a response was present absent or doubtful The evoked response recording was then subsequently reread by the author

In the patient group the 1 ¹/ to 2 ¹/ hour test session consisted of the presentation of sets of clicks ($N=100$) and pure tones ($N=50$) from 250 Hz to 6 000 Hz through a loud speaker which was mounted 30 from the baby s head The stimuli were presented at irregular intervals between 2 and 5 sec Responses to light flashes and a no stimulus control were also recorded for comparison with the auditory responses For the tones an electronic switch was adjusted to produce a stimulus whose duration was 100 msec A rise decay time of 10 msec was usually used but for some early recordings the rise-decay time

was 25 msec The test session for the no mal children differed som times from that for the patient since th emphasis was not so much on threshold d terminations as other evoked re sponse chara teristics The standard test con sisted of clicks at three in nsities and light flashes at three in nsiti s However many partial and compl t pure tone au I grams on this group were al o obtain d

During th first years of evoked response testing in our laborat ry a sound trea d t n environm nt was not availabl At pres nt a standard a ous ic test booth is being used

Only sound field stimulation was used for testing With th bird stimuli used standing waves wer not a troubl esom source of dis tortion The technician att mpt d t present the first set of stimuli well above thresh ld Stimuli were a nuated in 10 or 15 dB steps until th evoked response was no longer d tected at which poin th stimulus intensity was increased until a response appeared again Averaging was perform d using a CAT com puter and th responses read ou on line using a pen and ink plo er

RESULTS

Evoked response characteristics

Th auditorily-evoked EEG response consists of a series of waves which may last a second or more The most constant components occur within the first second after an auditory stimu lus but since infants responses tend to be slower than those of adults and since in sleep later response components may be accentuated a least one second of the post stimulus EEG should be analysed Som parts of the re sponse may under certain conditions be con taminated by extracerebral potentials from th muscles of th neck or from eye movements These do not present a problem in audiom etry Th auditory evoked response is usually maximal at th vertex but can also be easily detected from most of th frontal parietal and temporal scalp Its most prominent component during wakefulness and also during much of th course of sl p is a biphasic wave occurring between 80 and 300 msec after th auditory stimulus is given The prominent vertex posi tive component of this wave is usually d signated P (Fig 1) The intracerebral sources of th vertex response are not as yet fully elucidated Th y may not be responses of th primary auditory receiving areas (which are relatively inaccessible to scalp electrodes) but of association areas The appearance and rela tive prominence of each of th several com ponents of th response are individually in fluenced by many factors such as stimulus parameters age of th subject and his state of consciousness (Barnet & Goodwin 1965 Lenard et al 1967 Davis et al 1965 Rapin et al 1966 Barnet 1965 Weitzman et al 1965) Davis (Davis et al 1965) has em phasized the lack of close relationship of its characteristics to loudness or oth r psycho physical functions However despite the vari ability of th response in series where both EEG and voluntary thresholds were obtained from awake children over 3 years old and from adults the m asures were within a few dB of each other (Davis et al 1967) Part of an EEG audiogram is illustrated in Fig 2

In very young and uncooperative patients recording during sleep natural or sedated has several advantages 1) a long artifact free re cording session is possible without taxing the

The audiometer were cal brated using TDH 39 earphones with a B & K or General Radio sound level meter and an artificial ear free field readings at 30 inches from the speaker were also obtained The amplitude setting of the low frequency oscillator used to provide the tones was adjusted at the be ginning of each test session so that a steady 1000 Hz tone presented to the artificial ear attached ear phones produced a reading of 116 dB re 0.0002 dynes cm on the sound level meter this same tone produced a 78-80 dB free field reading at 30 inches from the loud speaker i e at the position of the subject's head When the duration of this stimulus was 100 msec i e the actual test stimulus it was an average of 75 dB above the psych ustic thresh old of 5 adults with normal hearing The strongest test stimuli at each frequency were adjusted so that they were 75-80 dB above the average psychoacoustic threshold of 5 normal hearing adults determined un der the recording conditions which were used for the patients

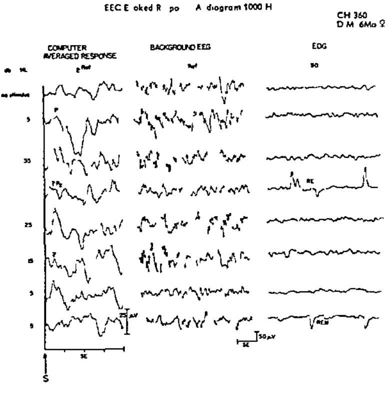
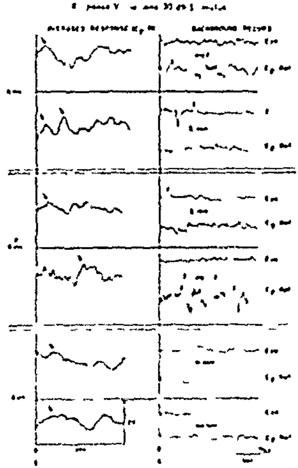


Fig 2 Part of an EEG audiogram of a 6-month-old girl showing normal response variability as the stage of sleep shifts

child 2) certain relatively easy to recognize response features are accentuated during sleep 3) response decrement with repetitive stimulus presentation does not occur during sleep (Johnson & Lubin 1967) as rapidly or to the degree that it does during wakefulness It is therefore important to become familiar with evoked response changes which are related to age state of consciousness and the sedative which is employed especially in evaluating responses which are near threshold Figs 2 and 3 show changes in the evoked response as the background EEG shifts During mixed frequency relatively low voltage REM sleep evoked response threshold is sometimes but not always raised During a very high voltage background EEG the signal noise ratio may be unfavorable and the response more difficult to extract from the high background To circumvent this difficulty a larger N may be averaged or else the stimulus may be repeated when the background EEG tracing returns to the moderate voltages of stage 2 sleep It is our impression that irregular intervals between stimuli rather than a constant interstimulus interval promote a higher amplitude response Reports differ on this point (Nelson et al 1969 Rothman & Davis 1969)

Response amplitude is directly related to interstimulus interval in fact a recovery period of 10 sec or more seems to be necessary to see maximum amplitude responses (Davis 1968) Very long interstimulus intervals are impractical because of the prolongation of the test session however intervals of 2 to 3 sec are usually sufficient Near threshold response detection can be improved by increasing the interstimulus interval

The responses recorded from the vertex electrode were larger than those from the left or right central electrodes (C_3 & C_4) The lateral electrodes were helpful in deciding in some cases where responses were doubtful Some of the patients showed left right response asymmetries these findings will be discussed in an



1 Responses of three subjects to ... presented at 30 dB HL. The arrows point to prominent ... features of the individual subject's response. The second response of subject PP is a fairly common variant at low stimulus intensity which appears usually in light sleep.

other report. The reference electrodes for C_z were placed on the mastoids or ear lobes, or on the occiput. In many subjects responses were clearer when one or the other reference was used. This was not predictable. The O-mastoid brainwave was also averaged; this provided a control for averaged brainwave activity at a site removed from the active electrodes. It would seem desirable, if the recording channels are available, to average two vertex derivations, one referred to the ear lobe or mastoid and the other referred to an occipital electrode. There was considerable variability in response form even in the normal infants, and in infants with brain abnormalities even more. Using several electrode combinations and observing the averaged brainwave patterns obtained during the presentation of several sets of stimuli often was helpful in the judgment as to whether a response was present or not. The agreement between the independent scoring of the technician and the author was high. About one-third of the patient group had evoked potential characteristics (other than elevated threshold) which were considered abnormal, e.g. extremely high amplitude, long latency, simple response form or prominent left-right asymmetry. The background EEGs of about one-fifth of the patients were abnormal. This apparently did not interfere with the evoked potential recording. However, no patient with a hypsarhythmic pattern was seen and no recordings were done during prolonged seizure discharges. Responses were elicited during the almost isoelectric phase of the newborn's trace alternant pattern, but in two hydranencephalic patients with flat EEGs no responses were seen. These two infants showed primitive startle responses to sound (Barnet et al. 1966).

Evoked responses in normal sleeping infants

In many young infants it was possible to detect responses during sleep at levels which were at or within a few dB of the adult psychoacoustic thresholds established for this study (cf. Fig. 2). In a significant percentage, however, stimulation even at suprathreshold levels did not yield responses on all trials. Table II gives the percentage of EEG responses obtained to 3 levels of stimulus intensity in 141 normal children. Sixty-three percent of the children were tested more than once. In about 20% of the tests up to 6 months of age responses were not definitely seen or were absent at the 50 and 35 dB levels (a few technically inadequate tests were not included). In most of the test sessions the response to only one set of stimuli at each level was averaged and no special effort was made to repeat presentations to which there was no response.

Repeating a stimulus to which a response had not been evident, either during the same or a

Table II *Computer averaged auditory evoked EEG responses (358 tests on 141 normal infants)*

Age group (months)	N Tests	65 dB +	65 dB ?	65 dB −	50 dB +	50 dB ?	50 dB −	35 dB +	35 dB ?	35 dB −
0–2	124	97 6	2 4		93 3	1 7	5 0	64 0	6 0	30 0
2–4	69	89 0	7 0	4	79 0	13 0	8 0	69 0	13 0	18 0
4–6	49	100			79 1	6 4	14 5	82 3	5 8	11 7
6–9	38	100			89 0	11 0		90 0	10 0	
9–12	23	100			95 6	4 4		100		
12–15	25	100			100			91 7	8 3	
15–18	9	100			100			100		
18–24	18	100			100			100		
24–36	5	100			100			80 0		20 0

In dB (ISO) above normal hearing adults psychoacoustic thresholds under the same recording conditions used for the infants
Stimulus Clicks *N* averaged = 100 Interstimulus interval = 2 5 sec

later test resulted in a significant increase in response detection (Rapin & Graziani 1967) The results of re testing are given in Table III

Of the 141 presumed normal hearing children who were tested 45 (32%) showed no response to a presentation of a 35 dB stimulus set on one or two presentations However if the test was repeated three times there were no normal subjects who did not show a response on at least one of the tests From the data in Tables II and III we conclude that validity of a finding of no or doubtful response is increased by repetition of the test stimulus and by testing when the patient is over 6 months

Since both behavioral (Hoversten & Moncur 1969) and EEG evoked response data in infants (Lenard et al 1969) suggest that there are differing levels of responsiveness depending on the quality (e g pure tones vs voice) and intensity of the stimulus additional normative data relating the type of stimulus its intensity and the age of the subject will be *important to increase the usefulness of EEG* audiometry as a quantitative test

A few children who had poorly defined responses on several tests are being followed to rule out hearing loss It is possible that a few babies had temporary conductive losses associated with middle ear pathology on some of the no response tests However babies with symptoms of URI were not tested and although URIs were actually more common after age 6 months than before responses were harder to obtain in the first half year of life In the newborns fluid or detritus in the middle ear may have resulted in conductive losses but this does not explain the high percentage of no and doubtful responses in the 2 to 4 month group Difficulty in EEG response detection at low stimulus levels in this age group has also been the experience of other laboratories Part of the explanation for high evoked potential threshold may lie in the fact that evoked potential amplitudes tend to be low in REM sleep Since in the early months of life REM sleep occupies a proportionately greater part of the sleep cycle the likelihood of stimulus presentation during REM sleep increases Another factor which may contribute to the higher percentage of positive responses in the older babies may be related to the fact that

Table III

Number of times subject was tested	N subjects	N subjects having no response at 35 dB on any test
Once	75	24 (32)
Twice	14	2 (14)
Three times	17	0
More than three times	35	0
Total *N* subjects	141	

evoked potential amplitudes in a given state of sleep increase slightly but significantly with age.

Since hearing losses of less than 30 dB have little effect on language development or the quantity of speech (Bakwin 1968) a method which will reliably test to within 20–30 dB of adult threshold is sensitive enough for use with infants. The current literature suggests that other testing methods for use in children under 1 year are not as sensitive as EEG audiometry (Hoversten & Moncur 1969).

Evoked responses in the patient group

One hundred children referred for a question of hearing loss received EEG audiograms on one or more examinations (see Table II). The largest single diagnostic entity was congenital rubella syndrome reflecting the epidemic of 1964. Nearly two-thirds of this group were under 1 year of age when first evaluated. The large majority of the patients had multiple physical and developmental problems. The following case studies are given as examples of the type of patients seen.

Case 1. E. I. Fifteen-month-old child with congenital rubella syndrome. Hearing loss was suspected by the foster mother from about 4 months of age. Bilateral cataract extractions were performed at 10 months and contact lenses prescribed. Growth and development were retarded. He had a [illegible] at 14 months [illegible] he [illegible] seemed to respond to [illegible] noise and whistles at 35 dB presented [illegible] by turning in the direction of the sound. Startle response was evident at 90 dB. His hearing was felt to be adequate for the acquisition of speech. EEG audiometry was performed at 15 months. Evoked responses appeared between 65 and 80 dB HL. At a re-evaluation by an audiologist at 17 months he did not respond at all to sound stimuli and was thought to have a severe bilateral hearing loss; a trial period of hearing aid use was recommended along with a nursery school program for the hearing impaired.

At 27 months he was again seen for evoked response audiometry. At this visit he weighed 24 lb and his head circumference was 47.5 cm (50 percentile for 11 mo). Gross motor development was fairly normal; he was visually handicapped and had no speech. The foster mother reported that although he was fairly easy to manage at home, he struggled and protested each morning when [illegible] inserted the contact lenses and the hearing aid [illegible] mold. She

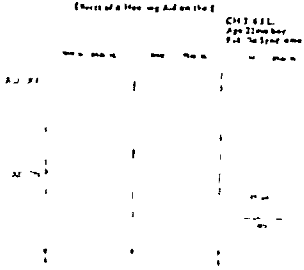


Fig. 4. Part of an audiogram performed on a 27-month-old boy (Case 1) wearing a Sonotone [illegible] hearing aid. The ear mold was left in place and the test was performed with the hearing aid alternately on or off.

saw little improvement in either his visual or auditory responsiveness with the aid. EEG evoked responses were recorded alternately with the hearing aid switched on or off. Part of the audiogram is shown in Fig. 4. Evoked responses were clearer when the aid was used. The behavioral and EEG findings were interpreted to mean that the patient's abnormalities in speech and language were caused by both peripheral hearing loss and CNS factors (i.e. mental retardation). It was felt that the child should be encouraged to wear the hearing aid to increase auditory input. This case illustrates our conclusion that in many children with multiple handicaps evoked response audiometry gives an earlier and more reliable assessment of hearing than clinical and behavioral observation.

Case 2. J. C. Eight-week-old boy, product of an uneventful pregnancy. Birth weight 7 lb 4 oz. The initial physical examination was normal. He showed normal weight gain. At 1 month his parents became extremely anxious about a possible hearing loss because of his lack of responsiveness to musical toys and the absence of startle responses to loud noises. His responses to voice were inconsistent. The parents tested his hearing repeatedly night and day. The pediatrician and an ENT consultant agreed that the baby's responses to sound were poor and were in contrast to his excellent visual alertness. There were

Several factors decrease the validity of testing with the hearing aid in the manner described, i.e. with the aid turned off a conductive loss is added by the ear mold; in free field testing the problems of distortions of the auditory input through the aid are increased.

no abnormal findings on physical examinations The EEG evoked response audiogram obtained at 8 weeks was normal Responses were present to pure tone stimuli at 20–30 dB HL from 500 Hz to 6 000 Hz. The parents were referred to the Hearing & Speech Center for clinical evaluation but did not keep their appointment because they began to see improvement in the child's auditory responsiveness Follow up at 1 year of age showed a normal child who babbled a good deal localized even faint sounds and spoke two words

This was a fat placid infant who showed a marked difference between auditory and visual responsiveness Although a month or two of patient waiting by the parents would have settled the question the EEG audiogram allayed their anxiety so that they could relax and enjoy their normal infant

In this infant, who showed normal growth and development and was visually alert the lack of behavioral responsiveness in the presence of a normal EEG audiogram suggests delayed maturation of audition with good prognosis However in an infant who is uninterested in his environment and/or who has signs of neurologic impairment a normal EEG audiogram would strengthen the possibility that a central nervous system abnormality e g mental retardation or aphasia was the cause of the auditory unresponsiveness (cf case 3)

Case 3 Y C Thirty four month old girl Slow development no speech and lack of interest in the auditory environment

The patient was the product of a full term normal pregnancy and delivery Birth weight was 7 lb Family history was negative for deafness She did well until 6 months of age at which time she developed tonsillitis and otitis media with a temperature of 105 F for 5 days There were no seizures or periods of unresponsiveness Because of meningismus a spinal tap was performed which was negative She seemed to recover completely but subsequent development was slow She sat at 10 months and walked at 17 months but gait remained broadbased and somewhat ataxic Aside from repeated episodes of tonsillitis and middle ear infections her health was good Hyperactivity temper tantrums and the relatively little interest she showed to people in her environment made her difficult to manage Shortly before 2 years of age the parents and the pediatrician suspected the patient was deaf because speech was not developing A neurologist examined her at 2 years and felt that she showed no evidence of progressive or focal neurological disease but that she was retarded and probably deaf She was seen on three occasions over an 8 month period in an audiology and speech clinic She was hyperactive and could not cooperate with testing In the sound field environment no consistent responses to signals in any modality were noted Occasionally there were minimal overt responses such as eye blinks or head turning to music and sounds of 50–60 db HL. Threshold could not be established The audiologist felt that auditory behavior was suggestive of a central auditory disorder and that peripheral hearing loss could not be ruled out During part of the time from age two to three she attended a nursery school for hearing impaired children At 2 years and 10 months EEG audiometry was performed High amplitude responses were seen to clicks from 65 dB down to 5 dB above adult voluntary thresholds Responses were also obtained to pure tones at 500 4 000 and 6 000 Hz down to about 30 dB The patient awoke before softer sounds could be presented The EEG audiogram was interpreted as essentially normal It was felt that peripheral hearing loss was not playing a significant role in the abnormalities of language and communication shown by the patient

The difficulties and uncertainties encountered in the clinical assessment of a patient of this sort are the rule rather than the exception In these patients EEG audiometry can make a substantial contribution by providing an early definitive assessment of peripheral hearing and thus make possible more rational planning for the patients habilitation

Case 4 A H Four month-old boy with rubella syndrome Born by Caesarean section to a 16-year-old unwed mother The neonatal period was complicated by poor temperature control and persistent hepatosplenomegaly and failure to thrive Cardiac anomalies (V S D) and microcephaly were present EEG evoked responses recorded at 4 months to clicks at 65 50 and 35 dB HL were clear but response latency was prolonged A psychologist observed responses to several auditory test items Because of unfavorable home conditions and his poor health the patient spent his first 4 years in hospitals or in convalescent homes He had bouts of congestive heart failure and pneumonia and middle ear infections At 2 years he was hospitalized for several months because of bacterial endocarditis At 2 years 3 months EEG evoked response audiometry was performed The child at this time babbled and used some gestures but had no speech and comprehension seemed limited He was a friendly child who accepted the often painful medical procedures without much resentment or protest A pediatrician thought he could hear but was mentally retarded Evoked responses were obtained to clicks at 65 and 35 dB HL. EEG response threshold for pure tones at 250 to 4 000 Hz was 60–70 dB HL Behavioral audiological evaluations were made when the child was 2 / years old He responded to noise and to music at 90 dB Responses were not improved after bilateral myringotomies for serous otitis media He was felt to have a severe bilateral hearing loss Hearing aid evaluation and a nursery school program were begun

At 4 years and 10 months of age EEG-evoked responses to sound were similar to those recorded at 2 years With the hearing aid responses seemed somewhat clearer This test was incomplete because the child awoke and became too active to proceed A clinical pure tone audiogram performed at 4 years showed an average pure tone loss of 102 dB ISO and a speech awareness threshold of 72 dB There were no responses (either ear) at the maximum output

of the audiometer at 500 and 1 000 Hz and at 2 000–8 000 Hz responses dropped out at 80 90 dB ISO

The nursery school teacher reported that the child could lip read and imitate several sounds but not words or phrases In group lessons he showed hyper activity and a very short attention span but he was very cooperative in individual drill and seemed alert and intelligent He was living in a good foster home and showing encouraging progress in physical and mental development

In this patient who was physically and developmentally retarded the relative contributions of rubella encephalopathy physical illness institutionalization and peripheral hearing loss were difficult to assess. His responses in behavioral audiological evaluations indicated profound loss which was probably due to the sum of peripheral and CNS auditory defect The EEG responses which probably measure the con tribution of peripheral defect were consistent with only a moderate loss Neither test measures hearing in this brain-damaged child in the sense that it is measured in the voluntary audiogram of a normal adult EEG evoked responses cannot always be re lated directly to psychophysical measures of sensation and perception In the brain-damaged patient there may be a pronounced dissociation between acknowl edgement by the brain of the receipt of sensory input i.e the evoked response and auditory sensation Ap plications of evoked response testing to the under standing of central disorders of audition i.e aphasia are a potentially fruitful area of research

Table IV indicates the degree of hearing ab- averaged across all frequencies found initial EEG test Moderate to severe loss was found in 47% of the group and normal hearing in 40%

All of the children suspected of hearing loss received one or more clinical appraisals of hearing and speech although the younger in fants were usually evaluated initially by a pediatrician neurologist or psychologist rather than by an audiologist Over three fourths of the group were evaluated at some time dur ing the study by an audiologist The overall percentages in Table IV for each category were similar for the first clinical evaluation except that in 20% of the cases there were no definite conclusions However when the in dividual test results for each child on whom a tentative clinical diagnosis had been made were compared about 25% were found to be in disagreement As was expected most of these tests were on brain damaged and mul tiply handicapped patients

Table IV

EEG Audiometry	Age (months) 0-6	6–12	12 18	18 24	24–36	Totals
Normal	20	10	3	3	4	40
Hearing loss						
slight						
(40 dB)	1	3	1	2	3	10
Moderate						
(0 70 dB)	6	6	4	2	3	21
Severe						
(70 dB)	12	5	6	1	2	26
*	1	1	0	0	1	3
	40	25	14	8	13	100

The test results obtained by the clinical evaluation and by EEG may in some cases not be directly comparable for two reasons the first having to do with the fact that the re sponse measures of the several tests used may reflect different aspects of the auditory process and the other methodological Be havioral responses to sounds may reflect a level of integration from the startle of the anencephalic which requires no forebrain to the voluntary responses of the 3 year-old dur ing the play audiogram which depend on cor tical integration and association areas EEG evoked responses while requiring functional auditory pathways to the level of the cortex (Barnet et al 1966) do not necessarily in dicate that higher perceptual processes are in tact Behavioral and EEG tests give comple mentary rather than identical pictures of the functional state of the auditory system It is important to keep in mind the level of auditory functioning which is being tapped by the test which is being used Methodological differences between the clinical and EEG testing also limit precise comparison of the results obtained Repetitive stimuli with sharply defined onsets are required for evoked response audiometry Speech music and a variety of calibrated noisemakers were usually employed in the clini cal test rather than pure tones In addition the measurements were obtained in several laboratories where different sound field con ditions prevailed and different instrumentation was used

During the period of follow up which was from 2 months to 4 $^1/_2$ years the results of the behavioral and EEG evaluations tended to converge (e g Case 1) In almost all cases where a clinical diagnosis could be arrived at the initial EEG diagnosis was confirmed A primary advantage of the EEG method seemed to be that diagnostic information was available early and most often through one test session The endpoint i e the evoked response in the EEG test was reproducible and could be validated by repeated checking In contrast for the behavioral evaluation of the hyperactive mentally retarded or withdrawn young patient whose behavioral responsiveness was erratic or fleeting and whose limit of tolerance to testing was low several visits over a lengthy period were almost mandatory

On approximately 8 % of the patients test results continued to differ during the period the patients were studied (e g Case 4) In 5 patients the clinical evaluation suggested poorer hearing than the EEG evaluation the reverse was true in the remaining three Five of the patients were grossly retarded the other three were rubella patients with several handicaps In 4 patients 3 of whom were under 1 year of age evaluation of hearing was done by a pediatrician or neurologist rather than an audiologist

One or more follow up evoked response recordings were done on 27 patients from 2 months to 4 years after the first test In 22 patients (82 %) test retest findings showed substantial agreement In 4 young patients with rubella syndrome follow up EEG examination showed higher evoked response thresholds this may indicate a progressive hearing loss in these patients The 1 patient who showed improvement in evoked potentials had 21 trisomy (mongolism) At 2 and 3 days of age he showed virtually no behavioral or EEG responses to sound Retest at 2 $^1/_2$ years showed only a moderate elevation of evoked response thresholds but behaviorally the child still seemed profoundly deaf

CONCLUSIONS

EEG evoked response audiometry was performed on 100 children under the age of 3 years referred for possible hearing loss Control recordings were done on 141 normal hearing children under three Most of the records were made while the children were asleep The normal children were not sedated the older patients were usually given a sedative

Evoked responses to auditory stimuli of moderate intensity were seen in all of the normal children Response forms were influenced by the type of background EEG the age of the subject and stimulus parameters Although variability both in and between subjects was considerable the agreement between scorers was good as to whether a response was present doubtful or absent

Responses at adult hearing threshold were detectable in some but not all recordings from presumed normal hearing infants About 30% did not show an averaged response to a single presentation of a set of clicks at 35 dB HL However all of the presumed normal hearing babies showed responses at 35 dB HL if tested three times Methodological changes e g lengthening of the interstimulus interval near threshold increased likelihood of response detection

Elevation of evoked response threshold was found in about half of the children in the patient group Many of the patients both those with and without peripheral hearing loss as judged by evoked response audiometry had severe mental or neurological handicaps which made assessment of their hearing by behavioral audiometry difficult or impossible Evoked response audiometry can often provide in one session information which may take using clinical methods repeated testing and observation over a period of months or years

Follow up EEG audiometry performed in 27 patients from 2 months to 4 years after the initial test showed a test retest agreement of 82%

Clinical evaluation of hearing and EEG

evoked response audiometry differed signifi cantly in their initial assessments in about 25% of the patients Over a period of follow up this disagreement was reduced to about 10% The patients involved were all brain damaged multiply handicapped youngsters

EEG-evoked response audiometry is a use ful and reliable means of testing hearing In young children and in mentally and neuro-logically handicapped children it can provide information not obtainable by other methods It is a laboratory test which should be used in conjunction with and should not supplant clinical audiological psychological and neuro-logical observation and assessment

The presence of the evoked response in dicates that sound has changed the ongoing electrical activity of the cerebral cortex it does not signify that conscious perception has occurred

Because of the expense and complexity of the equipment and the special training in volved both in its use and in the interpretation of the test results it will not be practical to use evoked response audiometry for auditory [illegible] tests However it will be of con [illegible]able value in pediatric medical centers and [illegible]al audiological units

ACKNOWLEDGMENT

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ZUSAMMENFASSUNG

EEG Audiometrie mit ausgelösten Antworten wurde bei 241 Kindern unter 3 Jahren durchgeführt von denen 100 Patienten zur Beurteilung des Hörver mögens eingewiesen waren und 141 Kinder nor mal hörten Insgesamt wurden 498 Aufnahmen meistens im Schlaf registriert Viele Kinder des Krankengutes hatten schwere geistige oder neuro-logische Defekte die die Beurteilung mittels klinischer Audiometrie erschwerte Audiometrie mit ausgelösten Antworten lieferte oft eine vollkommenere diagno-stische Auskunft eher als dies mit anderen Methoden erreichbar wäre Bei einigen normalen Säuglingen vor allem denjenigen unter 6 Monaten waren die Schwellen der ausgelösten Antworten im Schlaf er höht Eine Verbesserung der Messungen nahe der Schwellen wurde möglich durch Abwandlung der Registriertechniken Wir ziehen den Schluss dass EEG Audiometrie mit ausgelösten Antworten ein wertvolles Mittel zur Beurteilung des Hörvermögens bei jungen oder behinderten Patienten darstellt

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THE MUCOUS GLANDS IN CHRONIC SECRETORY OTITIS MEDIA

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Abstract From a patient with mild chronic secretory otitis media the mucous membrane of the osseous part of the Eustachian tube and the middle ear were removed *in toto* and stained by the PAS-alcian blue whole mount method whereby the mucous glands and goblet cells stain selectively This revealed a surprisingly large number of mucous glands as well as increased density and extent of goblet cells The shape structure size and distribution of the glands are described In the osseous part of the Eustachian tube 135 glands were found in the tympanic cavity 2 3 in the epitympanon 60 in the antrum 20 and in the mastoid process 50 glands average density 0 9 1 9 1 4 0 3 and 2 4 glands per square mm respectively The glands were found to be in different secretory stages 188 in an active 102 in a transitional and 198 in a degenerated stage each ng its characteristics All the glands are ab- al and responsible for the viscous mucous secre which may be present in chronic secretory otitis media The role of the mucous glands in the pathogenesis and course of chronic secretory otitis is discussed It is pointed out that the symptoms are due to the secretory capacity of the mucous elements tubal function and the ciliary function of the epithelium

Almost 100 years ago Politzer (1878) gave a thorough and still valid description of the clinical features and treatment of secretory otitis media In his *hydrops ex vacuo* theory he mentioned the aetiological role of tubal occlusion in the formation of the middle-ear secretion In the course of time there has been a great deal of discussion concerning the validity of this theory The most serious objections are that the middle ear secretion has such a high protein content (Ivstam 1954 Vuori 1959) that it must be interpreted as an exudate and that secretion may also be seen in the middle ear when the auditory tube is patent (Zollner 1942) or in the presence of a drum perforation and with a functioning drain Some workers (Blegvad 1941 Zollner 1942 Sadé 1966) feel that the pathogenesis must be more complex without wanting to deny that tubal occlusion is involved

In a systematic study of the tubal and middle ear mucosa in an autopsy material of adults and children we found one case with a large number of mucous glands in the middle ear mucosa Five years previously this patient had been subjected to otological examination because of secretory otitis media We feel that the combination of an ante mortem clinical examination and a post mortem histopathological study using a new method of preparation and staining justifies a detailed report in an effort to adduce new aspects concerning the aetiology and pathogenesis of secretory otitis media

PREVIOUS INVESTIGATIONS

Little has been published about the mucous elements of the Eustachian tube and middle ear in secretory otitis media or in other diseases of the middle ear

In chronic suppurative otitis Friedmann (1963) found PAS positive gland like structures or columnar epithelium in 30° of biopsies from the mastoid process removed at mastoidectomy

Bendek (1963) studied mucosal biopsies



Fig 1 Medial part of the mucosa from the osseous part of the Eustachian tube and the tympanic cavity with mucous glands The density decreases from the tympanic orifice (*O*) to the fourth (*4*) and third (*3*) quarter of the tube in the hypotympanon anteriorly (*HA*) the density is greatest on the promontory (*P*) slight in the meso-tympanon between the promontory and the prominence of the facial canal (*M*) there are large numbers of degenerated glands ×9

from the middle ears of 40 patients with secretory otitis media as well as from 35 patients with chronic suppurative otitis media In both groups about half had secretory epithelium and about half of these cases showed mucicarmine positive glands In biopsies from the middle ears of patients with serous otitis media Sade (1966) found distinctly PAS positive glands intraepithelial mucus producing elements and PAS positive secretion in the tympanic cavity Most authors (Zollner 1942 Eggston & Wolff 1947 Friedmann 1963) have found no mucous glands in the middle ear of normal adults or in newborns (Buch & Jørgensen 1964) or foetuses (Tos 1970 *a*) - Paparella & Lim (1967) during

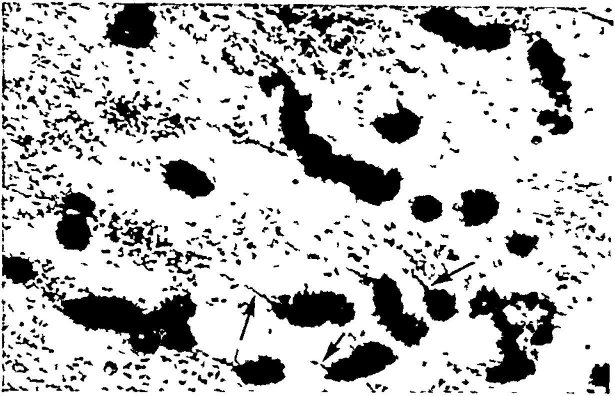


Fig. 2. Several active glands from the anteromedial part of the hypotympanon close to the tubal orifice. Most of the glands are simple tubular, two are branched showing 2–4 tubules. Goblet cells in the epithelium. From the glandular orifices streaks of mucus pass towards the pharynx (*arrows*). × 170.

5 patients with chronic secretory otitis media found cholesterol granulomas, simple tubular glands and major irregular cyst-like structures, in places lined with columnar epithelium.

Previous descriptions of mucous glands are based on studies of sections. A systematic study of the tubal and middle ear mucosa from a patient with secretory otitis media has not previously been done by the whole mount method. This method is far superior to the study of sections in respect to assessing the number, density and morphology of the glands as well as their distribution in the various parts of the middle ear.

MATERIAL AND METHODS

The patient was a 51 year old man who had been examined for impaired hearing in 1965. He gave a history of middle ear catarrh during childhood but had never had aural discharge. In 1962 he had consulted an otologist because of hearing impairment. He was treated with politzerization which had an immediate but short lasting effect. The hearing impairment had been accentuated during catarrhal conditions a couple of times each year.

At the objective examination in 1965 the drums were retracted. Audiogram showed a hearing impairment of the conduction type of 40 dB in the left ear and of 25 dB in the right. After politzerization the audiogram disclosed normal hearing in both ears. X-rays of the temporal bones showed reduced pneumatization on both sides and a large antrum. In 1970 the patient died of a metastasizing hypernephroma.

The left middle ear mucosa was fixed 3 hours after death, 4 ml formol alcohol being injected into the middle ear. The tubal mucosa as well as the mucosa of the tympanic cavity, epitympanon, antrum and mastoid process were removed *in toto*. As large a part of the mucosa



Fig 3 Great density of active glands anteriorly in the hypotympanon (*HA*) and in the tubal orifice (*O*) In the middle of the promontory (*P*) only a few glands In the superior part of the promontory and in the mesotympanon (*M*) the degenerated glands predominate on the epithelial surface plaques of mucus (*arrows*) × 15

as possible was removed and stained by the PAS alcian blue whole mount method (Tos 1970 *b*) (Fig 1) The preparations were examined under the stereomicroscope in a magnification of × 40 at which the shape distribution and situation of the mucous glands could be assessed and all glands counted

Parts of the mucosa from various segments of the middle ear and Eustachian tube were then cut into 5 μ serial sections and stained with haematoxylin eosin PAS alcian blue Feulgen nuclear staining and van Gieson staining whereby the mucosal structure and extent of the goblet cells could be assessed

RESULTS

In the mucous membrane of the osseous tube tympanic cavity epitympanon antrum and mastoid process there were numerous mucous glands of various shapes and sizes and in different secretory stages In the tympanic cavity there were moderate quantities of mucus On the epithelial surface in the tympanic cavity and tube a few streaks of mucus were seen These streaks began at the glandular orifices and passed towards the tube and pharynx (Fig 2) This direction of mucus transport is presumably a result of ciliary function in the Eustachian tube and middle ear More often there were major or minor plaques of mucus on the surface of the middle ear epithelium (Fig 3) Numerous goblet cells were present in the epithelium The goblet-cell density was particularly great in the osseous part of the Eustachian tube in the tubal orifice and in the anterior part of the hypotympanon (Fig 2) Towards the promontory and the posterior part of the mesotympanon the density decreased



Fig 2 Se... part of the... Most of ...

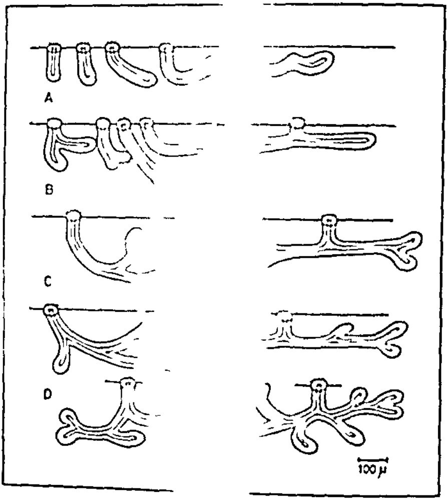


Fig 4 Gra... presentation of the shape ... and ... of the tubules in the active glands. Different varieties are shown (A) Simple tubules (B) Branched glands with ... tubules (C) Branched glands with 4 tubules ... Larger glands with ... tubules.

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Shape structure and size of g...

All glands were tubular consisting of one or more tubules but without acini. By shape and size the active glands may be divided into (*a*) simple tubules without branches (*b*) branched glands with 2 tubules (*c*) branched glands with ... tubules and (*d*) large glands with several tubules.

(*a*) Simple tubules (Figs 2, 4 *a*) consisted of only one tubule which was simultaneously the excretory duct debouching through an orifice on the mucosal surface. The length of the tubule ranged from 100–300 μ averaging 200 μ. Its diameter was 50 μ but somewhat narrowed immediately beneath the orifice. The tubular lumen was of the same diameter 20 μ throughout the length of the gland. The short tubules grew perpendicularly down in the mucosa, the longer ones always showed an oblique course.

(*b*) Branched glands with two tubules had one excretory duct at the end of which there were two tubules usually of equal length (Figs 2, 4 *b*). These glands were of two main shapes. Either the excretory duct was long measuring 150–300 μ passing obliquely down in the mucosa and ending in two short tubules, 100 μ in length. Such varieties were present predominantly in the tympanic cavity. In the other variety the excretory duct was very short as a rule only 50 μ coursing perpendicularly down into the mucosa and dividing immediately beneath the epithelium into two long, 200–300 μ tubules. These glands from 400–600 μ in length were present exclusively in the osseous tube and in the tubal orifice where they formed a characteristic appearance (Figs 1 ...



Fig 5 A large active branched gland with several tubules from the anterior part of the promontory × 205

all being distributed in the tympano-pharyngeal direction The diameter of the excretory duct and tubule and that of the lumen was the same as in the simple tubules

(*c*) Branched glands with four tubules have arisen when the gland primordium has divided twice so that two lateral ducts ending in two short tubules debouch into each excretory duct (Figs 2 4 *c*) Frequently these glands were 600 μ in length and of the same calibre as the other glands Just like glands with two tubules these glands also occured in two main varieties One with a long excretory duct and short tubules at the end This variety was encountered in the tympanic cavity The other variety had a very short excretory duct dividing into two long lateral ducts with two short tubules at the end This variety was present in the Eustachian tube

(*d*) Large glands with several tubules were the largest ones situated singly in the Eustachian tube and tympanic cavity The main duct had two lateral ducts into which several tubules debouched (Figs 4 *d* 5) Often the glands were symmetrical showing an equal number of tubules from each lateral duct The glands were frequently 700 μ long and the tubules usually numbered 6–8 Glands having more than 10 tubules were not observed

The varieted shapes of glands their distribution and size are determined by the space conditions available during their development and growth as demonstrated for the glands in the trachea (Tos 1966) bronchi (Tos 1968) and Eustachian tube (Tos 1970 *c*) When the tracheal glands develop during foetal life the mucosa and submucosa consist of fairly loose tissue so that the glands can grow freely and branch

The formation and growth of the abnormal glands in the osseous part of the Eustachian tube and in the middle ear takes place under entirely different conditions The mucous membrane is thin without any actual submucosa The vascular system nerves and fibrous tissue have already formed and there is but little space for the newly formed glands This explains why the majority are small with few tubules and why there is only a small number of branched glands

The glands were found to be in different secretory stages In active glands the ducts and tubules were lined with PAS alcian blue positive secretory epithelium The tubular lumen was narrow not dilated and there was no stagnation of secretion in the duct system (Figs 2 4 5) Degenerated glands showed considerable dilatation of the ducts and tubules (Figs 3 6) which were filled with stagnated mucus The secretory epithelium had entirely disappeared being replaced by PAS alcian blue negative epithelium simple in places

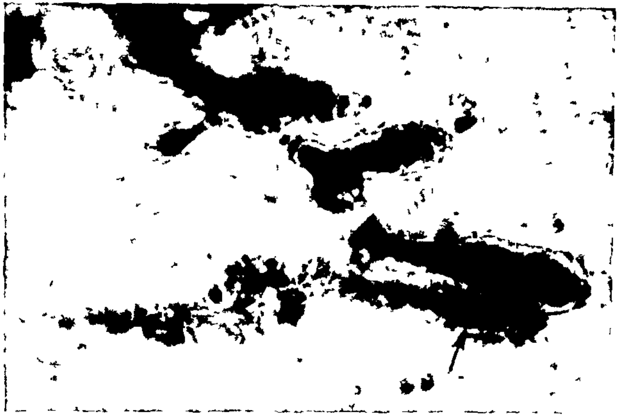


Fig. 6. Several degenerated simple tubules from the superior part of the tubal orifice. At the bottom a transitional variety (arrow) with incipient degeneration where the lumen is only moderately dilated and the secretory epithelium fairly well preserved. 00

ed in others. Between the two extreme types there were transitional varieties with a less dilated duct system and fairly well preserved secretory epithelium (Fig. 6). A definite relationship was found between dilatation of the ducts and the extent of secretory epithelium. The more dilated the ducts, the fewer mucous cells in the tubules.

Distribution, number and density of glands

The glands were counted in the entire mucosa and separately in the various parts of the tube and middle ear whose areas were measured. Thereafter the density of glands in each part could be calculated. In each part the number of glands in the various secretory stages was counted. In the 370 square mm of mucosa there

Table I. *Number and density of glands and area of studied mucosa. Number of glands in different secretory stages*

Locality	No. of glands	Area (mm)	Density of glands (per mm²)	Number of glands: Active	Transitional	Degenerate
Osseous tube	135	144	0.9	79	20	36
Tympanic cavity	223	116	1.9	95	52	76
Epitympanon	60	4?	1.4	5	15	40
Antrum	20	58	0.3	4	8	8
Mastoid process	50	20	2.5	5	7	38
Total	488	370	1.3	188	102	198

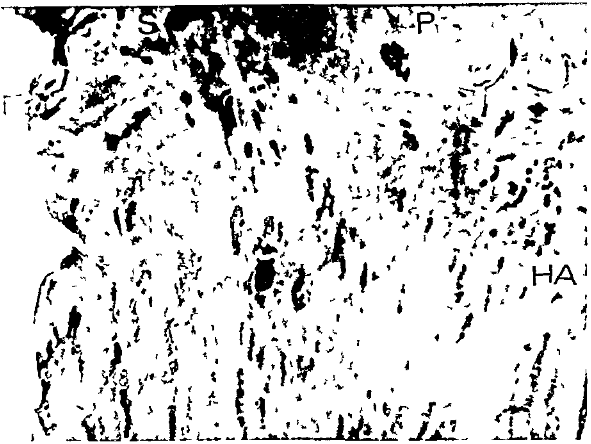


Fig 7 Medial part of the mucous membrane from the tympanic orifice and anterior part of the hypotympanon (*HA*) where the density of active glands is at a maximum Middle part of the promontory (*P*) with only a few glands and the superior part of the promontory (*S*) with a large number and density of degenerated glands as well as of transitional varieties ×25

were 488 glands with an average density of 1 3 glands/square mm (Table I)

Osseous part of the Eustachian tube

In the entire osseous tube there were 135 glands The density differed widely in the tympano-pharyngeal direction as well as between the medial and lateral wall In the tympanic orifice there were 71 glands (Table II) 63 of which were situated in the medial wall (Figs 3 7) In the lateral wall of the tympanic orifice there were only 8 glands lying close to the anterior edge of the drum (Fig 8)

In the fourth quarter—the Eustachian tube being divided into four equal quarters called the first (pharyngeal) second third and fourth (tympanic) quarter—there were 61 glands 57 of which were in the medial and only 4 in the lateral wall The majority of glands were situated close to the tympanic orifice the density decreasing considerably towards the tubal isthmus (Fig 1)

Four findings are characteristic of glands in the osseous part of the tube

(1) A marked decrease in density towards the isthmus where no glands were found (Fig 1) The ordinary mucous glands of the tube which average 86 in number in foetuses and prematures—and presumably also in adults (Tos 1970 *a* *c*) extend from the pharyngeal orifice to the middle of the tube In the present case the normal tubal glands also reached no further than the middle The unknown pathological irritant which had caused

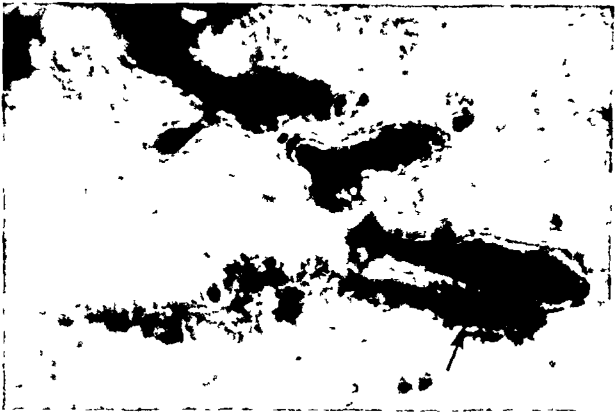


Fig 6 Several degenerated simple tubules from the superior part of the tubal orifice At the bottom a transitional variety (*arrow*) with incipient degeneration where the lumen is only moderately dilated and the secretory epithelium fairly well preserved ×200

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Antrum	20	58	0.3	4	8	8
Mastoid process	50	20	2.5	5	7	38
Total	488	370	1.3	188	102	198

epitympanon antrum and mastoid process as compared with the tympanic cavity and Eustachian tube

DISCUSSION AND CONCLUSION

On the basis of the history and the objective examination *ante mortem* it must be concluded that our patient had mild chronic secretory otitis media In the tympanic cavity there was on gross examination a small quantity of mucous secretion The examined pieces of mucosa from the Eustachian tube and tympanic cavity showed a considerable PAS alcian blue positive layer of secretion covering the mucosa indicating that the secretion was mucous Sections from various parts of the middle ear showed that the mucous secretion housed scattered lymphocytes and mast cells and that the submucosa was moderately thickened showing increased vascularization and small numbers of scattered mononuclear cells indicating a chronic catarrhal mucosal lesion

However the most surprising and most important finding was the large number of mucous glands in the various parts of the middle ear and the fact that these glands were in different secretory stages In addition goblet cells were far more widespread than usual being demonstrable in all parts i e as far back as the mastoid process True in the latter site the density was low unlike that in the osseous Eustachian tube and anteriorly in the hypotympanon where it was extremely great The findings suggest certain conclusions with respect to the pathogenesis of chronic secretory otitis media

The secretion sucked from the middle ear in secretory otitis media usually does not resemble either a transudate or an exudate It is tenacious thready and gelatinous and resembles mucus Indeed mucopolysaccharides have been demonstrated in this secretion by Zechner (1965) Moreover by PAS alcian blue staining of secretion from other patients with secretory otitis media we have found a typically blue staining indicating a high content of mucopolysaccharides Mucus cannot be the result of transudation or exudation only an active product of the demonstrated mucous glands and goblet cells in the tubal and middle ear mucosa

More rarely suction from the middle ear yields a serous straw-coloured fluid easy to evacuate and very unlike mucus The difference between the two types of secretion is grossly evident and very probably there is a question of two different manifestations of chronic secretory otitis On the one hand mucous secretory otitis media with active mucus producing glands and goblet cells and on the other hand serous otitis media with a thin fluid whose origin has not yet been definitely demonstrated and which cannot be definitely explained by the finding of mucous glands Sade (1966) has suggested that the serous secretion might be a product of serous glands in the middle ear but all the active glands in our case were purely mucous

The high protein content of the mucous and serous secretion (Ivstam 1954 Vuori 1959) as well as previous histological investigations (Zollner 1942) indicate that the mucosa is affected by chronic catarrhal changes The presence of mucous glands and the increase in the number of goblet cells with mucus production is one of the sequelae of this condition exudation presumably another

By infection and prolonged tubal occlusion the middle ear mucosa is influenced to metaplastic transformation of the original epithelium into ciliated columnar epithelium with goblet cells (Friedmann 1955) During this process mucous glands will form The action upon the mucosa must have existed a long time before the formation of glands sets in (Senturia et al 1962) Therefore months or even years may pass before the formation of secretion in the middle ear is sufficient to give rise to subjective complaints

Accumulation of mucus in the middle ear and the appearance of symptoms in secretory otitis media is an interplay of the following factors

(1) The presence of the glands and goblet cells and the production of mucus The more active the glands the more rapid the accumulation of mucus

(2) The secretory state of the glands Since the mucus production depends upon the number of active glands not of the degenerated ones, the amount of mucus will be determined by *the relationship between the two types of gland*

(3) The role of the Eustachian tube in transporting the mucus away from the middle ear If the tube is fully functioning the newly formed mucus may be transported away If tubal function is compromised the mucus will stagnate the symptoms worsen and possibly the secretory activity of the glands will increase

(4) The role of ciliary function in transporting the mucus to the rhinopharynx In catarrhal conditions the ciliary function may be affected so that stagnation of mucus occurs in the middle ear

Cessation of the secretion—which in cases without adhesive otitis is tantamount to a cure of the secretory otitis media—may be explained by the histological appearances which show ly degenerated inactive glands and no or only a very few active mucous glands

The present findings aid in the understanding of the pathogenesis and clinical features of chronic secretory otitis media The topography of the glands and the differences in density show that a mucosal biopsy has to be removed anteriorly in the hypotympanon or close to the tympanic orifice if it is to afford an impression of the number of active glands and thereby of the severity of the disease A mucosa biopsy from the promontory which is technically more easily accessible is of less value as in this site the density of glands is low

ZUSAMMENFASSUNG

Von einem Patienten mit leichterer sekretorischer Otitis wurde die Schleimhaut des knochernen Teils der Eustachischen Rohre und des Mittelohres im ganzen freipraparlert und nach den PAS-Alcianblau methoden der Ganzpraparate gefarbt Dabei wurden die mukosen Drusen und Becherzellen selektivt dargestellt Man fand überraschend viele muköse Drusen sowie eine grosse Becherzelledichte und Ausbreitung Form Struktur Grosse und Verteilung der Drusen wurde beschrieben Im knochernen Teil der Eustachischen Rohre wurden 135 Drusen gefunden in der Pauke 223 in Epitympanon 60 im Antrum 20 und in der Mastoidwurzel 50 Drusen mit einer Dichte von je 0 9 1 9 1 4 0 3 und 2 4 Drusen/mm² Die Drüsen wurden in verschieden Sekretionsstadien gefunden 158 in den aktiven 102 als Übergangsform und 198 in den degenerativen Stadien jedes Stadium mit bestimmten Charakteristiken Alle beschriebenen Drusen sind pathologisch und produzieren das zahe mukose Sekret das bei der kronischen sekretorischen Otitis vorhanden ist Die Bedeutung der gefundenen Drusen für das Verstandnis der Pathogenese und des Verlaufes der sekretorischen Otitis wurde diskutiert. Es wurde unterstrichen dass die Symptome der sekretorischen Otitis von der Sekretionskapacitat der mukosen Elemente der Tuba und Ciliefunktion des Epithels abhangig sind

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HISTOCYTOCHEMICAL PATTERN OF ENZYME DISTRIBUTION IN COCHLEA OF THE KANGAROO RAT (*D SPECTABILIS*) AT REST AND FOLLOWING AUDITORY STIMULATION

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(Received February 2 1971)

Abstract The histocytochemical distribution of enzymes in the cochlea of the kangaroo rat *Dipodomys spectabilis* at rest and following auditory stimulation was investigated At rest enzyme distribution in *D spectabilis* resembles that previously reported by Webster & Stack (1966 1968) in *D merriami* Krebs cycle enzymes are prominent in the sensory epithelium stria vascularis spiral ligament and external spiral sulcus The spatial pattern of general esterase activity is also similar No increase or decrease of either succinic dehydrogenase or carboxylic esterase activity as measured by a microscopically visible quantitative change in deposition product is observed following acoustic stimulation Injury to the basal and second turns however is seen in a few cochleae The validity of using histochemical methods for the demonstration of fluctuation in enzyme activity is discussed

The relative activity of selected enzyme systems in the cochlea at rest and following auditory stimulation has commanded the attention of several investigators (Vosteen 1960 Koide et al 1960 Vinnikov & Titova 1964) an interest prompted by the question of what role certain enzymes and their associated substrates play in the transduction and transmission of auditory stimuli in the organ of Corti particularly in the hair cells Vosteen (1960) and Vinnikov & Titova (1964) report a decline in succinic dehydrogenase activity in the outer hair cells after various parameters of stimulation while Koide et al (1960) observed a similar decrease limited to the stria vascularis

The present paper describes results obtained in a histocytochemical investigation of the pattern of enzyme distribution in the cochlea of the banner tailed kangaroo rat *Dipodomys spectabilis* both at rest and following auditory stimulation The histologically modified organ of Corti of the kangaroo rat and its maximal range of auditory sensitivity have been previously noted (Webster 1961 1962 1966)

Cochlear distribution of respiratory enzymes and carboxylic esterases in Merriam s kangaroo rat *Dipodomys merriami* has been reported by Webster & Stack (1966 1968) Except for a high concentration of non-cholinergic esterases in the cells of Hensen and higher succinic dehydrogenase activity in the external and internal hair cells the pattern of enzyme activity and their cochlear distribution is otherwise similar to that found in the guinea pig (Plotz & Perlman 1955 Vosteen 1960 1963 Chou 1961 Gerhardt 1961 1962 Spoendlin & Balogh 1963 Vinnikov & Titova 1964 Conti & Borgo 1965 Nakai 1965)

MATERIAL AND METHODS

Thirteen male adult banner tailed kangaroo rats (*Dipodomys spectabilis*) weighing between 100 and 130 g were selected for use The animals were anesthetized and stimulated in

This research was supported by USPHS grant no NB 05800 to DBW and NIH Predoctoral Fellowship no GM 13 394 to CRS

Table I *Parameters of stimulation for experimental animals*

Animal no	Frequency (Hz)	Intensity (dB)	Time
DS 4161	500	131	2 hours
DS 4137	1 500	95	1 hour
DS 4180	1 500	122	2 hours
DS 4131	2 000	98	30 mins
DS 4134	2 000	102	1 hour
DS 4154	2 000	118	1 hour
DS 4183	2 000	118	1 hour
DS 4076	2 000	120	20 mins
DS 4185	2 000	124	1 hour
DS 4171	2 000	131	1 hour
DS 4135	3 000	114	1 hour
DS 4181	Control		
DS 4020	Control		

the manner previously described (Stack & Webster 1970)

Pilot studies to determine the minimal sound pressure level required to produce a visible histochemical change in enzyme activity in the cochlea were conducted Pure tones of 1 500 2 000 and 3 000 Hz at sound pressure levels ranging from 98 to 131 dB were employed Stimulation time varied from a minimum of 20 min to a maximum of 2 hours (Table I)

Immediately after excitation the animals were decapitated their cochleae quickly removed and suspended in a Tris buffered 10% solution of EDTA at 4°C for 30–48 hours with constant stirring Following chelation the cochleae were placed in O C T embedding compound (Lab Tek) under vacuum for 5 min and then quick frozen in an International Harris Cryostat Model CT kept at −20 C 14 μm sections were cut and mounted on glass slides The activity of selected enzyme systems was then determined utilizing the following techniques succinic dehydrogenase according to Nachlas et al 1957 glutamate malate and lactate dehydrogenase as per Hess et al 1958 cytochrome oxidase (Burstone 1960) acid and alkaline phosphatase (Gomori 1941 1950) ATPase (Padykula & Herman 1955) cholinesterase (Gomori 1952) and general carboxylic esterases according to the naphthyl acetate method of Gomori (described by Pearse 1960) and Holt & Withers technique (1952) utilizing o acetyl 5 bromo indoxyl as substrate Stained sections were studied with a light microscope Enzyme activity was determined by the amount of deposition product at any given site and rated on a + + + to 0 scale Photomicrography was done using a Zeiss Photomicro scope on those sections of particular interest

RESULTS

The histochemical distribution of selected enzyme systems in the cochlea of *D spectabilis* at rest resembles that reported for *D merriami* (Webster & Stack 1968) and is summarized in Table II Staining for ATPase is not as strong as reported previously but the regions of concentration remain the same The capillary walls in both the modiolus and stria vascularis show a heavy deposition of reaction product as do the nuclei of Hensen s cells

During the course of testing for glutamate and malate dehydrogenase it was discovered that if the slides were not post fixed after incubation staining in the sustentacular and border cell complexes deepened This accounts for the apparent discrepancy in the reactivity of these structures in *D merriami* and *D spectabilis*—sections from *D merriami* were immediately post fixed those from the banner tailed kangaroo rat were not

Two classes of enzymes succinic dehydrogenase and carboxylic esterase were tested for in the eleven stimulated animals The selection of these two enzyme systems for initial testing was prompted by the former s position as a key enzyme in the energy producing process of oxidative phosphorylation and the latter s candidacy for involvement in the transduction process (Webster & Stack 1968) Succinic dehydrogenase activity in the kangaroo rat cochlea is illustrated in Fig 1 carboxylic esterase distribution in Fig 2 Only two cochleae in the experimental group showed a lessening of deposition product indicating a loss in succinic dehydrogenase activity This occurred in the fourth turn and was particularly

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Carol R. Sack and D. B. Webster

From the Department of Biology, New York University, Bronx, N.Y., USA

(Received February 2, 1971)

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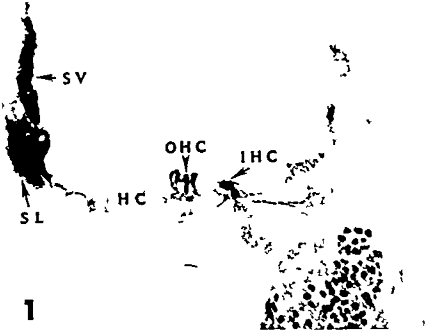


Fig 1 D spectabilis cochlear duct third turn succinic dehydrogenase activity *HC* Hensens cells *IHC* inner hair cell *OHC* outer hair cells *SL* spiral ligament *SV* stria vascularis × 155

hair cells In the third turn of the cochlear duct the section assumes a normal appearance

The pattern of histological injury to the organ of Corti as related above was repeated to a greater or lesser degree in eight cochleae DS 4131 lt DS 4134 lt and rt DS 4137 rt DS 4180 rt and lt DS 4183 lt and DS 4171 rt The sequence of disruption appears to be as follows In mild cases the delicate cells of Claudius are fragmented and consequently Hensen s cells lose their resting place and are found lying unattached within the scala media Next Deiters cells are seen to be damaged or absent and the outer hair cells either missing altogether or one or two (usually the inner most) still attached to the reticular lamina Disappearance of the pillar cells follows and lastly the inner hair cells and sustentacular cells Except for its limitation to the basal and second turns there is however no detectable correlation between the point of maximum disruption to the organ of Corti along the basilar membrane and the stimulus frequency nor can the variation in time and intensity of excitation be related to the degree of damage Furthermore cytological injury to the basal turn of the cochlea can be seen in individual sections cut from the two control animals The damage however is spotty and generally cannot be followed from one section to another

DISCUSSION

The histochemical distribution of selected enzyme systems in *D spectabilis* affirms the prominence of Krebs cycle enzymes in the sensory epithelium stria vascularis spiral ligament and external spiral sulcus previously reported by Webster & Stack (1968) in *D merriami* The spatial pattern of general esterase distribution is also similar

Even though numerous workers have demonstrated the presence of acetylcholinesterase in the vicinity of the hair cells (e g Schuknecht et al 1959 Spoendlin & Gacek 1963 Vinnikov & Titova 1964 Kaneko & Daly 1968) the present investigation on the kangaroo rat failed to do so Previous attempts to show its presence in the cochlea of *D merriami* and the guinea pig were also unsuccessful (Webster & Stack unpublished data) In all previous work care was taken to include a section cut through either the stapedius or tensor tympani muscle Strongly positive cholinesterase activity



Fig. 2. D. [illegible] transection of cochlea and [illegible] state esterases. U1 upper basal (first) turn, L2 lower second, U2 upper second, L3 lower third, U3 upper third, L4 lower fourth. Organ of Corti missing in upper part of basal turn, disturbed in lower second [illegible]

is always observed in the motor end plates of both these muscles a fact which confirms validity of the technique. We must therefore conclude that either no true cholinesterase is present in the organ of Corti or that it is present in insufficient quantities to be demonstrable by the histochemical techniques used

The results obtained in that part of the research dealing with a probable fluctuation in succinic dehydrogenase and general esterase activity following acoustical stimulation are not in accordance with those reported in the guinea pig by Vinnikov & Titova (1964) even though in some instances experimental animals were treated similarly. The results are more in agreement with those of Koide et al (1960) particularly with respect to dehydrogenase activity in the outer hair cells. Koide and his associates reported that after stimulation at 4 000 Hz 105 dB for 15 hours no change in enzyme activity is seen even though examination of the mitochondria reveal abnormalities. They did however report a fall-off in activity of the enzyme in the stria vascularis something which is not recognizable in the kangaroo rat

Vosteen's long term experiment (1960) are not repeated as such in the present investigation however considering Koide's results (Koide et al 1960) it is probable that what Vosteen witnessed as a fall off of enzyme activity in the outer hair cells after stimulation for 2 days was a reflection of extensive damage to the cells mitochondria a situation which would render the dehydrogenase non functional

Further consideration of the biochemical

characteristics of enzyme systems does not make the apparently negative results in the present investigation too surprising Theoretically it may be assumed that if the transduction process in the hair cells requires the availability of a high energy source (ATP) it would be expected that oxidative metabolism involved in the biosynthesis of ATP would be taxed under conditions of intense or prolonged stimulation The demonstrable Krebs cycle enzyme succinic dehydrogenase should be worked to capacity If the high activity of carboxylic esterases is in any way concerned with the transduction process then these enzymes would also be taxed under stress conditions

It can be imagined that this taxation or functional stress on the system could manifest itself in several ways If biological conservatism operates on a cellular level in the same manner as in the total organism chances are that not all reactive sites on the succinic dehydrogenase and carboxylic esterases—or any other enzymes for that matter—are being utilized under normal physiological conditions Continual stress on the system would result in more and more of the reactive sites becoming substrate linked until a point of saturation is reached Another possibility is that reserves are present as apoenzymes which can readily be converted to an active form under stres

The effect of such a step-up in the availability of reactive sites on the histochemical demonstration of enzyme activity must be considered An important point concerning the evaluation of relative enzyme activity in the hair cells or any other structure within the cochlea is that it is always tested for indirectly that is to say activity is measured in terms of a visible quantitative difference in deposition product the deposition product representing a combination of one of the reaction products from the biochemical degradation of exogenous substrate with a capture reagent Measured in these terms if more reactive sites are made available the amount of deposition product would be expected to increase if the enzyme is not saturated with substrate and tied up in an enzyme substrate complex at the time of staining If the latter were true a decrease in deposition product would be noticed and the situation incorrectly diagnosed as a decrease in enzyme activity One might also envision a situation in which some of the available reactive sites remain open and others linked with substrate such that the total reaction product (and subsequently the deposition product) appears the same as in a control animal This situation would also be incorrectly interpreted as no change in enzyme activity It must also be realized that the quantitative increase or decrease in deposition product must be sufficient to be visually apparent

Except for the work of Vinnikov & Titova (1964) all reported demonstrations of a decrease in enzyme activity following acoustical stimulation were in animals which had been exposed to moderate sound intensities for a prolonged period of time (e g Vosteen 1960 Koide et al 1960) In view of Beck s experiments on RNA and protein content in the outer hair cells prior to and post stimulation (Beck 1965) the decrease in enzyme activity may be considered to be a condition prompted by a general denaturation of cellular protein Beck reported a reduction in proteins and RNA in the external hair cells concurrent with structural modifications in their nuclei characteristic of nuclear stages in cytolysis

In the present work it is felt that experimental conditions are not solely responsible for structural damage to the organ of Corti in the lower turns In order to preserve enzyme activity the tissue is not fixed and the cells are subjected to freezing prior to cutting and thawing before staining There is also considerable compression of all cellular components of the organ of Corti during sectioning

Those cells to which processing is [illegible] deleterious are the delicate cells of Claudius resting on the basilar membrane [illegible] Hensen s cells Frequently they are found [illegible] missing their disappearance [illegible] or osmotic imbalance during [illegible] any event the net result [illegible]

stability of Hensen s cells which are now held in place mainly by their attachment to the reticular lamina In light of this it is understandable that cellular components of the basal turn (where the cells of Claudius are most prominent) should be disrupted occasionally in control animals Under these circumstances it is very difficult to ascertain in frozen sections the extent of injury to the organ of Corti caused by auditory excitation alone The uncertainty also accounts for the difficulty in correlating extent of damage to the cochlea with intensity of the sound source

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ZUSAMMENFASSUNG

Unter ... die histochemische Verteilung der Enzyme in den Schnecken des *D ...* im Zustand der Ruhe und nach Reizung der Gehörnerven Im Zustand der Ruhe war die Enzymverteilung ähnlich der wie sie von Webster und Stack (196?, 1968) in *D ...* beschrieben wird Krebs Zyklus Enzyme sind führend im Cortischen Organ Stria vascularis Ligamentum spirale und Sulcus ... spiralis ... Muster der allgemeinen Esterase Aktivität ... Weiter eine Steigerung noch eine Verminderung von succinodehydrogenase oder carboxylase Enzymen gemessen an der mikroskopisch sichtbaren quantitativen Veränderung des Ablagerungsprodukt konnte nach akustischer Reizung beobachtet werden In einigen Schnecken wurde jedoch eine Beschädigun der ersten und zweiten Windung beobachtet Die ... Gebrauchs der histochemischen Methode um Schwankungen in der Enzym-Aktivität aufzuzeigen wird diskutiert

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MICRORADIOGRAPHIC STUDIES ON THE OTOSCLEROTIC STAPES

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Abstract The distribution of mineral salts was studied in 58 normal and 80 otosclerotic stapedes of different degree of activity By means of soft roentgen microradiography it was possible to compare the distribution of mineral salts in otosclerotic foci and normal stapedes In the otosclerotic stapedes it was found that the active and quiescent foci are less mineralized than the parts not affected by the disease the foci are also less mineralized than the normal stapedes The active foci showed a lower X ray absorption than the quiescent ones this means that the otosclerotic foci increase their mineral content in the course of their evolution

Although the histological chances of otosclerosis the human stapes have been extensively studied and well described the data on the mineralization of the otosclerotic focus are still scanty and contradictory

The global mineral content of the auditory ossicles in normal and pathological conditions was biochemically evaluated by Maurer (1961–62) by a photon beam absorption technique by Evens & Henkin (1969) and by neutron activation analysis by Soifer et al (1970 *a*)

Soft roentgen ray microradiography of undecalcified sections of bone tissue has facilitated precise evaluation of the distribution of mineral salts at a microscopical level (Amprino & Engstrom 1952 Amprino 1952) This method was applied to the otosclerotic bone tissue by Danic et al (1963) Clarke (1964), Rockert et al (1965) and Meurman et al (1968) and gave contradictory results The present work was carried out in order to obtain further knowledge on the distribution of mineral salts in stapedes from patients with otosclerosis

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Part of this study was reported at the VII Workshop on Inner Ear Biology held in Stockholm June 1970

MATERIAL AND METHODS

In the present study normal (58 stapedes from cadavers ranging from a 6 months fetus to aged adult) and pathological materials (80 stapedes or fragments removed during stapes surgery for relief of a conductive deafness diagnosed pre-operatively as clinical otosclerosis) were examined

The stapedes were fixed in alcohol To compare the distribution of mineral salts in otosclerotic and normal stapedes groups of 9 stapedes were embedded simultaneously in methacrylates ground by hand to a thickness of 40–60 μ and then microradiographed The thickness of each section (including 9 stapedes) was measured and the difference between the sides of the same section resulted to be less than 10° In some groups otosclerotic stapedes were placed side by side to normal stapedes from cadavers of the same age (Figs 1 2) and in

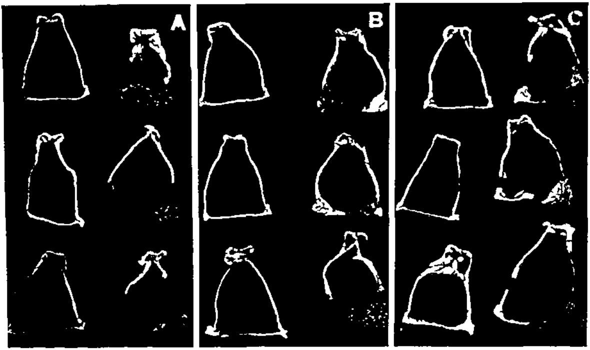


Fig 1 (*A C*) Microradiographs of groups of stapedes embedded simultaneously In each section three otosclerotic stapedes are placed side by side with normal stapedes of the same age ×6

others only pathological stapedes from patients of different age and clinical story This procedure permitted us to compare in the same microradiograph the mineral content of the otosclerotic foci with that of normal stapedes and to study the mineralization of foci having a different degree of activity (Fig 2) To obtain the microradiographs the sections were placed alongside a step-wedge of aluminium films (7 14 21 28 35 42 49 56 μ thick) on a Lippmann Gevaert maximum resolution film and pressed in contact with it The microradiographs for the present study were obtained with a Machlett type AEG 50T X ray tube (7 8 kV 18 mA filter 1 mm Be) Under these conditions the wavelength was 2 5–3 A which is the position of higher absorption coefficient for calcium

RESULTS

In our material an attempt was made to correlate the degree of mineralization with the activity of the focus The evaluation of the activity of the focus was based upon the dimensions of the marrow spaces the number shape and dimensions of the lacunae and the characteristics of the bone matrix

The more active foci show a great porosity due to an increase in the size and number of vascular spaces in the bone (Fig 3 *A*) These spaces are characterized by an irregular outline The number of osteocytes is also greatly increased There is a great variation in size of the lacunae within which the osteocytes reside which instead of being flat are very often oval or roundish in shape (Fig 4 *B*) The distribution of the lacunae is rather irregular Sometimes the lacunae are outlined by an area of low mineralization and seem to coalesce (lacunae confluentes Figs 3 *A* 4 *B*) The mineral content of the bone matrix is rather homogeneous The bone matrix is more radiotransparent than in the footplates of the controls and in the normal parts of the otosclerotic stapedes (Figs 2 5) When the focus does not affect the whole footplate bony fragments of normal aspect and remnants of calcified cartilage can easily be identified because of their

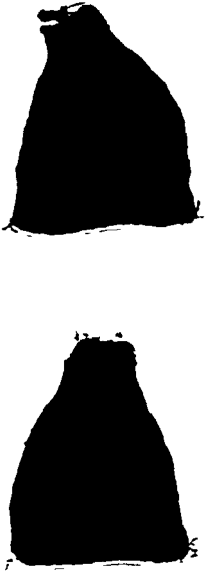


Fig 2 Microradiographs of stapedes in Fig 1 *B* at higher magnification × 17 2 (*A*) Normal stapes from a cadaver 34 years (*B*) Otosclerotic stapes removed *in toto* from a patient 35 years (C) Normal stapes from a cadaver 19 years (*D*) Otosclerotic stapes removed *in toto* from a patient 21 years In (*B*) and (*D*) the otoscl rotic process involves all the footplate except for small remnants of cartilage (*arrows*) easily identified for their high X ray ab orption Half of the footplate is involved with an active focus and the other half with a less active (*D*) or a quiescent one (*B*) The active foci show a lower X ray absorption

much higher X ray absorption (Figs 2 and 5 *A* *C*)

In the less active foci vascular spaces are less numerous and smaller and show a more regular outline (Fig 3 *B*) The bone shows a decrease in the number of osteocytes and in some areas a tendency to a lamellar arrangement (Fig 4 *C*) Some of the lacunae are flat

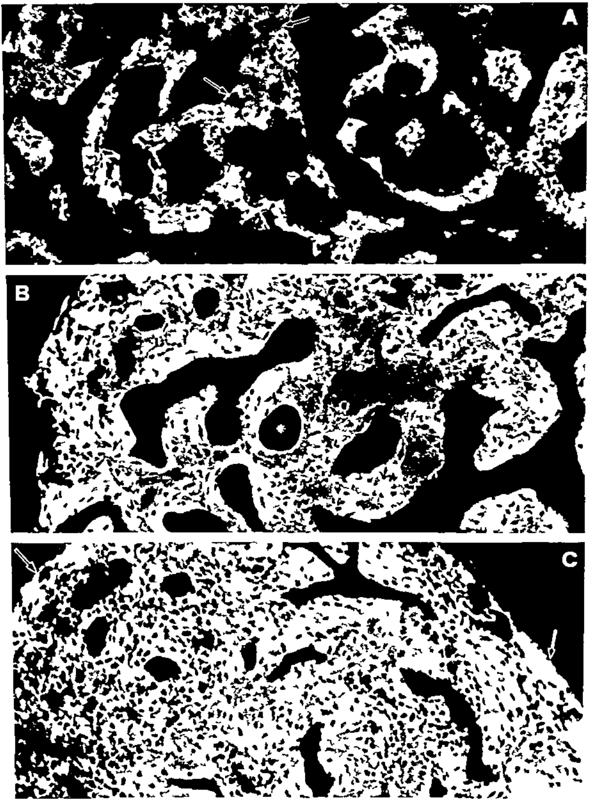


Fig 3 Microradiographs of an active (*A*) less active (*B*) and quiescent (*C*) focus × 150 In (*A*) arrows point to lacunae confluentes In (*B*) asterisk i a surface showing a high X ray absorpt sclerosis) In (*C*) arrows point to re the mineral content of the bon quiescent focus (*C*)

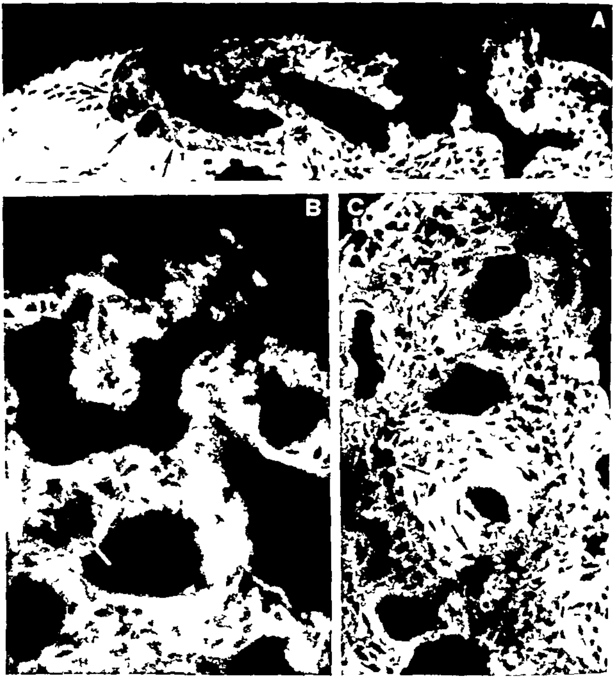


Fig 4 (*A*) Microradiograph of a marginal otosclerotic focus 45 years Arrows indicate an area of new bone deposition ×175 (*B*) Microradiograph of an active focus Arrow indicates lacunae confluents outlined by an area of low mineralization 51 years 350 (*C*) Microradiograph of a less active focus demonstrating Haversian sy tems with a different decree of mineralization 37 years Arrows indicate lacunae confluentes outlined by an area of low mineralization ×275

others roundish and show a tendency to coalesce (Fig 4 *C*) In comparison with the controls also in these foci the bone matrix shows a lower X ray absorption However the mineral content is not homogeneous in the different parts of the focus The areas of new bone deposition show a lower mineral density and run parallel to a smooth surface (Fig 4 *A*) Oc

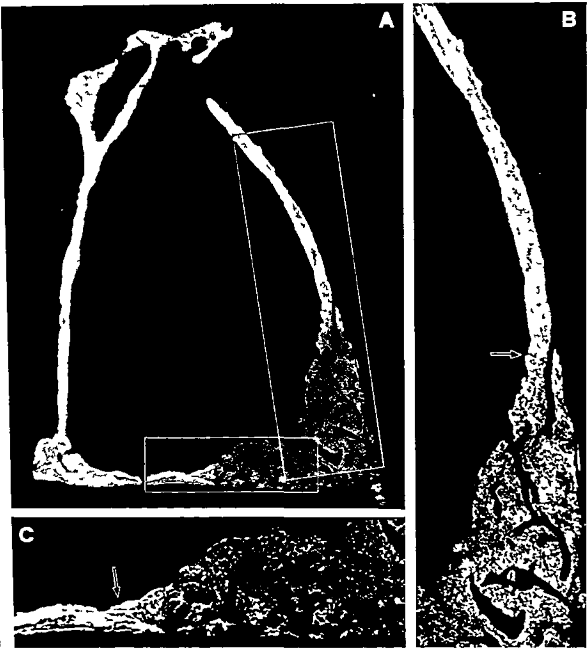


Fig 5 (A) Microradiograph of a stapes removed in toto from a patient with an active crural anterior otosclerosis 39 years × 35 The outlined areas are showed at higher enlargement in (B) and (C) (B) Otosclerotic focus involving the lower third of the anterior branch × 65 (C) The normal part of the footplate shows a higher X ray absorption × 80 In (B) and (C) arrows indicate the limit between the otosclerotic focus and the normal part of the stapes

casionally an osteoblast surrounds itself with bone matrix coming to reside in a lacuna and transforming in osteocyte Where new bone formation has been taking place but has recently stopped the surface of the bone becomes more highly mineralized and produces an edge sclerosis or ring of high mineral density surrounding the Haversian canal (Fig 3 B) Be

sides these smaller variations of the mineral content are evident in all the bone matrix (Fig 3 *B*) Small remnants of normal footplate or parts thereof not involved by the otosclerotic focus are easily identified as they have a higher X ray absorption and a normal histological appearance

In the quiescent foci the number and dimension of the vascular spaces are markedly reduced (Fig 3 *C*) The lacunae are not so numerous as in the active foci but much more abundant than in the normal stapes Haversian systems can frequently be observed The mineral content of the bone matrix is rather homogeneous *The X ray absorption of the bone* matrix is higher than in the active foci but it does not reach that of the normal stapes (Fig 2)

DISCUSSION

The data presented indicate that the X ray absorption of both active and quiescent foci is constantly lower than that of the normal stapes (Fig 2) and of the normal parts of the otosclerotic stapes (Fig 5) In our experimental conditions the inorganic part of bone accounts most of the total amount of radiation absorbed in roentgens Differences in the absorption of the X rays are therefore due to differences in the content of mineral salts This means that the mineral content of the otosclerotic bone is lower than that of the normal bone

There is a difference in X ray absorption between active and quiescent foci (Figs 2 *B* *D* and 3) The active foci show a lower X ray absorption which means that they are less mineralized than the quiescent foci and that in the course of their evolution the otosclerotic foci increase their mineral content

In the otosclerotic bone the number of osteocytes/mm³ has been evaluated to be around 110 000 compared to 20 000 in the lamellar bone and 50 000 in the non otosclerotic fibrous bone (Frost 1962) The presence of numerous large osteocyte lacunae could account for a low value of X ray density besides the calcium content of the bone matrix (Jowsey 1964) To be sure that this was not the case in our material we tested the microhardness of some normal and otosclerotic stapedes by making imprints on the interlacunar matrix The results obtained indicated that in the otosclerotic bone the microhardness was lower than in the normal bone which confirmed the interpretation we gave to our microradiographs

In the active foci we observed a great deal of enlarged lacunae surrounded by areas of low mineralization Sometimes the enlarged lacunae seemed to coalesce The pictures were similar to those found in hypoparathyroidism by Jowsey (1963) *in hyperparathyroidism by* Belanger et al (1963) in fracture callus by Duriez et al (1965) in rickets by Steendijk et al (1966) We are not in the position to say if the areas of low density in the active otosclerotic foci are representative of deep sealed bone resorption (osteocytic osteolysis) or of poor mineralization The first hypothesis could be supported by the electron microscopical finding of lysosome like bodies in the osteocytes of the otosclerotic focus (Chevance et al 1969) and by the large concentration of acid phosphatase histochemically (Albernaz & Covell 1961) and biochemically (Soifer et al 1970 *b*) demonstrated in the otosclerotic tissue

Several investigators (Danic et al 1963 Clarke 1964 Rockert et al 1965 Meurman et al 1968) have attempted to evaluate the mineral content of the otosclerotic bone with microradiography but with conflicting results According to Danic the otosclerotic bone is less mineralized than the normal and this was also confirmed by us Clarke s results seem to indicate that there are differences in mineralization depending on the age of the patients but his microradiographs are questionable Rockert found that the otosclerotic foci showed a higher mineral content compared to the healthy parts but we were not able to confirm this

Our observations on the mineral content of the stapes are in agreement with the measurements of Maurer (1961–1962) who found a decrease of calcium and phosphorus in extracts

obtained from ossicles from patients with otosclerosis Evens & Henkin (1969) found the same using a photon beam absorption technique Instead according to Soifer et al (1970 *a*) calcium and phosphorus concentrations determined in otosclerotic stapes by neutron activation analysis do not seem too different from those of normal stapes At the moment the reasons for this inconsistency remain unexplained

In Spain stapedectomies were carried out by L Garcia Iban z and in Italy by E Bo ca and S Iurato

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ZUSAMMENFASSUNG

Untersuchung der Mineralsalzverteilung an 58 normalen und 80 otosklerotischen Steigbugeln mit unterschiedlichem Aktivitatsgrad Unter Anwendung einer historadiographischen Methode konnte die Verteilung der Mineralsalze in otosklerotischen und normalen Steigbugeln verglichen werden An den otosklerotischen Steigbugeln wurde festgestellt daß aktive und ruhende Herde weniger mineralisiert sind als nicht von Otosklerose befallene Areale diese Herde sind auch weniger mineralisiert als normale Steigbugel Die aktiven Herde zeigten geringere Rontgenstrahlen absorption als die ruhenden dies bedeutet daß der Mineralsalzgehalt der otosklerotischen Herde im Laufe ihrer Entwicklung zunimmt

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OTOSCLEROSIS OF THE OVAL WINDOW NICHE AND THE CHOICE OF STAPES SURGERY

A Human Temporal Bone Study

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Abstract The localization and extent of the oto sclerotic foci in the oval window niche were stud ied in 66 human temporal bone specimens The bones were cut in serial sections of 20 microns According to the size of the otosclerotic focus and the degree of stapes fixation the material is divided into three groups The possibility of stapes surgery in each group is discussed

Some years after the re introduction by Rosen (1953) of the stapes mobilization technique for surgical treatment of otosclerosis Ruedi & Spoendlin (1957) reported the histological findings in 79 temporal bones with oval window otosclerosis in order to correlate the extent of stapes fixation with the probability of suc cessful stapes mobilization They came to the conclusion that mobilization would not be pos sible in 14 of these 79 cases Forty one of them were suitable for a mobilization procedure but the remaining 24 were borderline cases and the chances of a successful mobilization were very doubtful Although there were no audio metric findings and it was possible that some of the cases with so called fibrous fixation had no hearing loss at all these speculations on the basis of the histological findings were considered to be compatible with surgical ex perience in stapes mobilization operations

However in the last ten years surgical tech nique in oval window otosclerosis has changed markedly and instead of mobilization there are now two different techniques which are widely used to restore hearing

(1) Stapedectomy which utilizes a prosthe sis as a replacement for the fixed stapes (Some surgeons use the head and crura of the stapes instead of the prosthesis after removing the fixed stapes footplate and covering the oval window with a vein graft)

(2) The piston technique which consists of the drilling of a small hole in the stapes foot plate with a diamond drill after removal of the superstructure of the stapes

Both of these procedures have many techni cal variations Again it was Ruedi who in re examining the same material used in his work with Spoendlin tried to determine on the basis of the histological findings the pos sible success or failure of stapedectomy in the treatment of oval window otosclerosis (Lindsay et al 1963) He concluded that the original 14 cases considered as unsuitable for the mobilization of the stapes footplate would also present certain difficulties with stapedectomy He maintained that a good postoperative result with permanent hearing improvement could be expected in only 4 of the 14 cases In 3 of these the reclosure of the oval window after stapedectomy seemed probable in another 4 stapedectomy would be technically possible

Table I *Localization of the otosclerotic focus and its relationship with the mobility of stapes foot plate in 66 cases*

1 Otosclerotic focus in oval window region without fixation of stapes footplate	
Subgroup (*a*) oval window niche walls	6
Subgroup (*b*) stapes footplate only	2
Subgroup (*c*) oval window niche with fibrous changes in annular ligament but without bony fixation on the footplate	10
Total	18
2 Otosclerotic focus with bony fixation of the footplate	
Subgroup (*a*) the footplate is fixed only with a bony bridge or there is only partial involvement with otosclerotic bone	33
Subgroup (*b*) the footplate is fixed thickened and almost completely involved by otosclerotic bone	6
Subgroup (*c*) otosclerotic bone obliterates the oval window niche	9
Total	48

but there could be a great risk of inner ear damage According to him it would be im possible to perform a stapedectomy on the 4 cases with very thickened footplate and compression and subluxation of the stapes On the clinical material in this same series Lindsay et al (1963) estimated that this group represents probably less than 10 of clinical otosclerosis

In another series Gerlach (1963) found in 900 stapedectomies only 4 2% with oblitera tion of the oval window niche with otosclerosis and 12 7% with thickened stapes foot plate

Meanwhile the piston technique was in troduced by Shea (1960) for the first time in 1959 This method seems to be the procedure of choice in obliterative type of oval window otosclerosis

MATERIAL AND METHOD

Because of changes in technique on the one hand and the lack of audiometric data in the reports of Ruedi & Spoendlin (1957) and Ruedi (see Lindsay et al. 1963) on the other it seemed that it might be useful to review the temporal bones with oval window otosclerosis which we had in our collection in the Otopathology Laboratory at the Massachusetts Eye and Ear Infirmary in Boston The correlation was made between the histological findings the hearing loss (most of the patients had audiograms or some information about their hearing loss) and with the choice of operative procedure

Among the 450 temporal bones in this col lection 66 were found to have an otosclerotic focus in the oval window region The localiza tion and extent of the otosclerotic focus was estimated in serial sections of 20 microns The slides reproduced in this work represent only the most informative of the serial sections Most of the bones were cut in a horizontal plane though some were cut in the vertical In a few cases one side was cut in horizontal sections and the other side vertically The temporal bones used for histological analysis were re moved mainly from patients with some ear pathology or from patients with diseases which could possibly have some resulting ear pathol ogy and did not represent a typical sample of the population For this reason no statistical conclusions could be drawn from this material regarding the incidence of otosclerosis with relation to sex age or race Consequently only the histological findings are tabulated (Table I) For the same reason the cases in Groups I and II are unrelated especially since most of the cases in Group I had no hearing loss Only in subgroups (*a*) (*b*) and (*c*) in Group II could there be any relative significance but the numbers were too small for any profitable statistical evaluation

COMMENTS

In the first group (18 cases) the otosclerotic focus was localized in the walls of the oval window niche or in the stapes footplate was no bony fixation of the stapes



Fig 1 T L. 86 years Progressive sensorineural hearing loss and dementia in the last few years of her life Histology The stapes footplate is involved with otosclerotic bone but there is no involvement of the annular ligament or otic capsule There is no fixation of the stapes There is severe degeneration of the spiral ganglion in the basal 20 mm of the cochlea and a moderate degeneration of the organ of Corti in all turns with complete atrophy in the basal 3 *mm*

and in subgroups (*a*) and (*b*) the annular ligament looked normal There was no evidence of conductive hearing loss in any of these cases and therefore there was no indication for surgery In some of the cases the otosclerotic focus was overhanging the footplate but even then there was no hearing loss The 2 cases of isolated otosclerotic foci in the stapes footplate correspond to the cases first described by Guild (1944) (8 cases) Ruedi & Spoendlin (1957) also mentioned four cases of isolated stapes otosclerosis and Fowler (1957) one case The thickness of the stapes footplate in our cases had apparently no influence on sound transmission and conductive type of deafness was not evident in any of these cases However the development of an isolated otosclerotic focus in a stapes without connection with the otic capsule was strong evidence against the theory of the influence of mechanical factors in the etiology of otosclerosis (Šercer 1961) (Fig 1)

In subgroup (*c*) there was also no bony fixation of the stapes but the annular ligament looked narrow with more or less of an increase of fibrotic tissue The stapes itself was some



Fig 2



Fig 3



Fig 4 B A 44 years The patient had had a hearing loss from the age of 26 T audiogram shows a sensori neural deafness for high fr quences with a loss of 50- dB above 1 000 Hz for air and bone conduction Histo ogy The anterior margin c the oval window niche is o cupied by a focus of oto- sclerotic bone The annular ligament is partially involve and the articulation rim of the stapediovestibular joint irregular The stapes footpl is compressed and slightly displaced out of the vestibul There is no bony fixation of the footplate The organ of Corti is missing in the basal 15 mm of the coch ea and th spiral ganglion cell popula tion is reduced to 50 in th same area

Fig 2 P A 59 years This patients experienced mild hearing loss for some years The audiogram reveals normal bone conduction with air conduction threshold at 25-15 dB for low and middle frequences *Histology* There is an otosclerotic focus involving the otic capsule anteriorly to the oval window and overhanging the stapes footplate The annular liga ment in this area is narrowed and replaced mostly by fibrous tissue There is no bony fixation of th stapes The inner ear sense organs and spiral ganglion are normal

Fig 3 L G 60 years This patient was known t be deaf for many years and was wearing a hearin aid There is no audiogram availabl but the Webe test was negative with a 512 tuning fork. These find ings suggest that there was a hearing loss for a least 40 dB with a conductive component in lo frequences Histology A large otosclerotic focus in volves the entire otic capsule superior and anterio to the oval window The otosclerotic bone bulge into the anterior aspect of the oval window and dis places the anterior margin of the footplate slightl out of the oval window The annular ligament is narrowed anteriorly and partly replaced by fibrous tissue The articulation rim of the footplate is ir regular although there is no ankylosis The organ of Corti the vestibular sense organs and spiral ganglion are normal

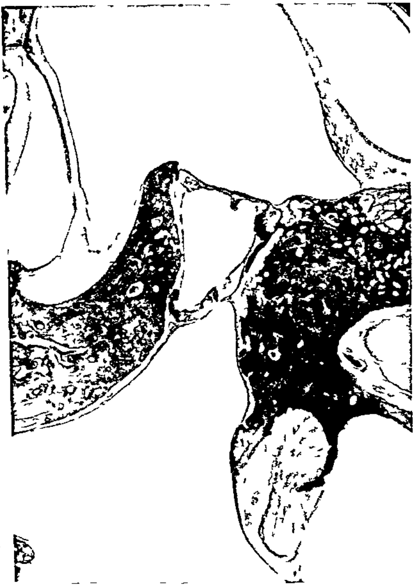


Fig 5 G N 53 years There was no history or hearing tests available Histology Examination reveals extensive otosclerosis involving the promontory and the otic capsule superior to the oval window niche The otosclerotic bone had proliferated to overhang the oval window and has wedged the stapes into a deep narrow niche only 0 6 mm large The stapes itself is not involved with otosclerosis but the annular ligament is narrowed and largely replaced with fibrous tissue The inner ear sense organs and spiral ganglion are normal

Fig 6 F S 61 years Bilateral hearing loss since the age of 40 Audiometrical testing revealed a conductive hearing loss with an air conduction threshold at the level of 50-70 dB and a bone conduction threshold at the level of 20-40 dB At the age of 55 an unsuccessful attempt at stapes mobilization was performed Histology There is a large otosclerotic focus anterior to the oval window The posterior part of the footplate is slightly displaced into the vestibule and the anterior part is ankylosed to the oval window wall by a thin bridge of otosclerotic bone There is moderate post mortem autolysis of the sense organs and although hair cells can be identified an accurate assessment of the population is not possible There is some loss of spiral ganglion cells in the lower basal turn

Fig 7 H A 56 years There was progressive hearing loss beginning at the age of 13 The audiogram reveals a bone conduction threshold at 10-25 dB and an air conduction threshold at the level of 60-70 dB Histology There is a large otosclerotic focus involving the otic capsule the anterior wall of the oval window niche the anterior half of the footplate and the base of the anterior crus The posterior part of the footplate is normal and free of otosclerotic bone The inner ear sense organs and spiral ganglion are normal

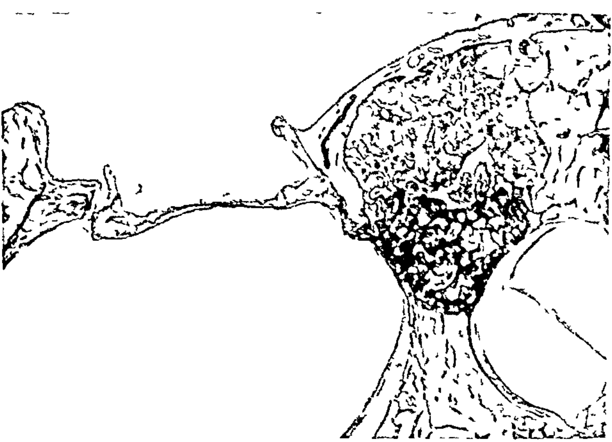
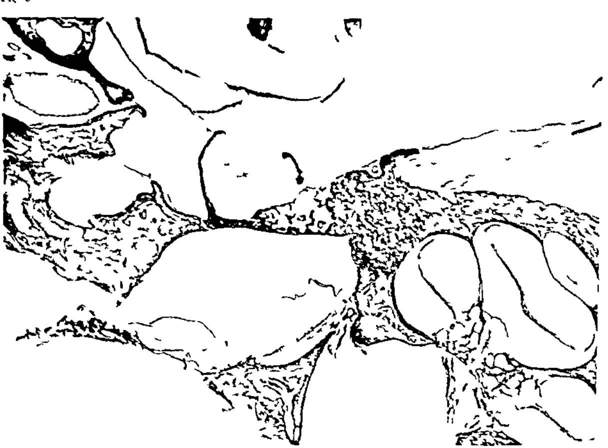


Fig 6



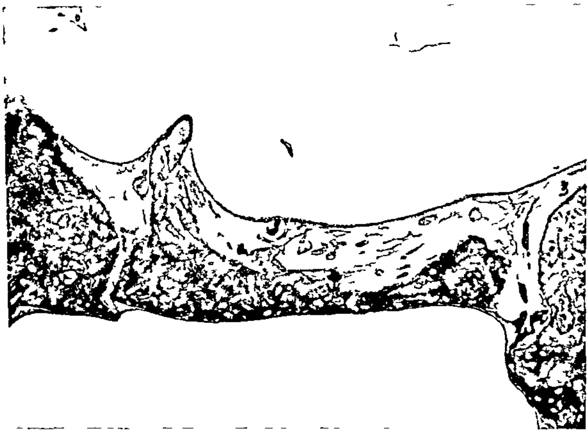


Fig 8 A F 53 years The patient had experienced progressive hearing loss over a period of 30 years Audiometric studies revealed a combined sensori neural and conductive hearing loss with the bone conduction threshold at 5-55 dB and air conduction threshold at 55-90 dB Histology There is a large otosclerotic lesion involving the entire otic capsule and the stapes The otosclerotic focus bridges from the footplate to oval window margins The footplate is completely involved by otosclerotic bone The remnants of the annular ligament can be seen but they are widely replaced by fibrous tissue Th thickness of the footplate is 0 3-0 6 mm The sense organs of the inner ear and the spiral ganglion are normal

times tilted and subluxated in the vestibule or compressed Lindsay et al (1963) classified this group of cases as fibrous fixation However it is impossible to evaluate the mobility of the stapes footplate on the basis of histological findings In 5 of our cases the hearing was normal in 1 there was no data in 2 there was a conductive hearing loss and in 2 sensorineural hearing loss The conductive hearing loss was slight in 1 case but in others there was an air–bone gap of at least 20 dB or more (Figs 2 3) However it must be emphasized that even in cases where there was compression of the stapes footplate and fibrosis in the annular ligament the hearing loss cannot be automatically attributed to otosclerosis because it could have quite a different etiology (Fig 4) Where surgery was indicated stapedectomy was in all of these cases the procedure of choice The only exception was if the otosclerotic focus involved extensive parts of the walls of the oval window niche narrowing the access to the stapes footplate In these cases drilling of the oval window niche walls would be necessary and a piston technique would have to be taken into consideration Fortunately such cases are rare and there was only 1 case in our material (Fig 5)



Fig 9 S M 66 years The patient had experienced a slowly progressive hearing loss since his youth and was using a hearing aid on his left ear He was known to have had a sister and a brother with earing losses Histology There is an otosclerotic ion involving the walls of the oval window niche e stapes and the otic capsule anterior to the oval window There is a complete circular ankylosis of the footplate and no remnants of annular ligament can be seen The footplate and the bases of the crura are greatly thickened by otosclerotic bone The thickness of the otosclerotic bone in oval window niche is 0 8–1 5 mm The sense organs of the inner ear are normal There is a 20% loss of spiral ganglion cells in the basal 10 mm of the cochlea

In the second group (48 cases) there was bony fixation of the stapes footplate (clinical otosclerosis) The material was divided into three subgroups (*a*) (*b*), and (*c*) according to the extent of the fixation Conductive hearing loss was always present but there was no close relationship between the magnitude of the hearing loss and the extent of the otosclerotic involvement of the stapes

In subgroup (*a*) (33 cases) fixation of the stapes was caused by a bony bridge between the otosclerotic focus and the stapes footplate (Fig 6) In some cases the footplate was fixed and partially involved with otosclerotic bone but even in these cases a part of the stapes *footplate (usually the posterior) was free* and unaffected by otosclerosis (Fig 7) Stapedectomy was in all these cases the most suitable procedure because a total or partial removal of the footplate could be effected without difficulty and as a rule closure of the air-bone gap could be achieved

In subgroup (*b*) (6 cases) there was complete involvement of the stapes *footplate* with otosclerotic bone The footplate was fixed anteriorly and posteriorly and only remnants of the annular ligament which was replaced by fibrous tissue could be seen (Fig 8) The thickness of the footplate varied in our series of cases between 0 3 and 0 9 mm (Table II)

Table II *Thickness of the stapes footplate in cases with complete involvement of the footplate with otosclerotic bone*

Case	Thickness of footplate (mm)
1 N M (left temporal bone)	0 3–0 5
2 N M (right temporal bone)	0 5–0 6
3 H R (right temporal bone)	0 5–0 7
4 A F (left temporal bone)	0 3–0 6
5 A F (right temporal bone)	0 5–0 9

Stapedectomy could be performed in all these cases In some of them it proved necessary to thin the footplate by using a drill and to perforate the footplate before removal with a hook

In the last subgroup (c) (9 cases) there was complete obliteration of the oval window niche by otosclerotic bone The otosclerotic foci in the oval window niche walls and in the stapes footplate became a uniform structure of otosclerotic bone and only occasionally could some remnants of the annular ligament be recognized (Fig 9) The average thickness of the otosclerotic bone in the oval window niche was 0 7–2 0 mm (Table III) In these cases only the piston technique could be used after drilling away the major part of the otosclerotic bone Hearing is not always completely restored and usually the air–bone gap is not closed because of fibrosis around the piston and then eventually a secondary fibrous fixation of the prosthesis to the oval window niche walls (Lindsay 1961)

Ruedi pointed out the dangers of surgery in cases with a thickened stapes footplate or with obliterative otosclerosis because of the possibility of cochlear duct damage and the dislocation of parts of the stapes footplate into the vestibule However the incidence of severe sensorineural hearing loss after stapes surgery is now less than 1° (Portmann & Sizen 1966 Schuknecht) Until now there was no histological evidence of a cochlear duct injury after stapedectomy

In 10 specimens of human temporal bone Subotic & Kaufman reported (1971) that the structures in the vestibule were surprisingly resistent to surgical trauma The displacement of a part of the stapes footplate to the vestibule or bony dust in the vestibule seem to have no influence on the postoperative improvement of hearing Also in a case where blood entered the vestibule during the operation not only was there no deterioration of the hearing but 18 months after the operation there was no damage of the membranous labyrinth and no evidence of blood could be demonstrated histologically In only 2 out of 10 cases examined after stapedectomy was there new bone formation in the region of the oval window (3 and 4 years after operation) There was no tendency to reclosure of the fenestra

On the other hand Kaufman & Schuknecht (1968) reported that the major cause of sensorineural deafness following stapedectomy is reparative granuloma and that the inner ear structures are only secondarily involved

CONCLUSIONS

In oval window otosclerosis without bony fixation but with fibrous changes in the annular ligament slightly tilted stapes and sometimes compressed stapes footplate (otosclerosis with fibrous fixation so-called by Ruedi) the conductive hearing loss occurs only occasionally and does not usually justify surgery The more severe hearing loss in these cases has to be

Table III *Thickness of the otosclerotic focus in cases with obliterative otosclerosis of the oval window niche*

Case	Thickness of otosclerotic focus (mm)
1 W N (left temporal bone)	0 9–1 10
2 W N (right temporal bone)	0 9–0 19
3 A S (right temporal bone)	1 2–1 9
4 B A (left temporal bone)	1 3–2 0
5 B A (right temporal bone)	1 6–2.0
6 L A (left temporal bone)	0 8–1 6
7 M A (left temporal bone)	0 7–1 3
8 M A (right temporal bone)	0 8–1 4
9 S R. (right temporal bone)	0 8–1 5

attributed to other causes (sensorineural hearing loss) If surgery is indicated, stapedectomy can be performed without difficulty

In cases with bony fixation of the stapes footplate stapedectomy is the procedure of choice Only in cases where access to the stapes footplate is extremely narrowed by otosclerotic foci in the oval window niche walls does the piston technique have to be taken into consideration

In cases with obliteration of the oval window niche by otosclerotic bone the piston technique is the best choice though the results are often unsatisfactory because of the connective tissue reaction around the prosthesis after drilling out the otosclerotic bone

New growth of otosclerotic bone on the edges of the surgical defect after stapedectomy occurred in only 2 out of 10 cases 3 and 4 years after operation The foci of new bone did not interfere with the mobility of the fat-wire prosthesis This confirms the clinical experience that reclosure of the oval window after stapedectomy is extremely rare

ACKNOWLEDGMENT

I am indebted to Dr H F Schuknecht for his kindness in permitting me to study the temporal bone specimens in the collection of the Massachusetts Eye and Ear Infirmary

ZUSAMMENFASSUNG

Die Lokalization und die Verbreitung der Otosklerotischen Herden in der Gegend des ovalen Fensters bei 66 humanen Schlafenbeinen wurde studiert Die Knochen wurden in Serienschnitten von 20 μ geschnitten Nach der Grosse der Otosklerotischen Herden und nach der Stufe der Stapesfixation wurde das ganze Material in drei Gruppen verteilt Die Moglichkeiten der operativen Eingriffe wurden fur jede Gruppe diskutiert

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HEARING DEFECTS IN MALES WITH SEX CHROMOSOME ANOMALIES

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Abstract Hearing was studied in 26 clinically and cytogenetically analysed men with different types of sex chromosome anomaly usually 47 XXY (Klinefelter s syndrome) The number of hearing threshold impairments of probably endogenous origin was unusually high (19%) In addition several cases demonstrated small but distinct dips in the middle frequency range This finding is of interest since this specific type of hearing threshold pattern is also recorded in nearly half of the cases with another sex chromosome anomaly Turner s syndrome Furthermore a similar threshold configuration is found in presumptive carriers of autosomal recessive genes for deafness In contrast to the patients with Turner s syndrome pathologically high stapedius reflex thresholds were recorded in 35% of the present material

The etiology of deafness often remains unknown since hereditary and acquired forms can only rarely be distinguished by current audiometric techniques There are however strong reasons to believe that there is a significant genetic background in cases of an unknown etiology

The relatively rare type of deafness with an apparently dominant mode of inheritance usually presents no difficulty in diagnosis since the audiometric pattern and the age of onset of the hearing loss often bear a close resemblance between affected relatives of different generations In contrast to these is the incomparably largest group having a recessive type of inheritance and in these cases the cause of deafness may be extremely difficult to diagnose Recent studies have shown however that presumptive heterozygotes can be detected by special audiometric methods (Anderson & Wedenberg 1968) Small but distinct dips in the middle frequency range of the hearing threshold and/or abnormally high thresholds for the acoustically elicited stapedius reflex were recorded in a very large proportion of such presumptive gene carriers

Patients with Turner s syndrome often demonstrate a hearing threshold pattern similar to that found in presumptive carriers of recessive genes for deafness (Lindsten 1963 Anderson et al 1969) This finding is of special interest since Turner s syndrome is caused by well-defined numerical and structural X chromosome aberrations resulting in short stature ovarian dysgenesis and various other anomalies (reviewed by Lindsten & Fraccaro 1965) In the present paper we report the finding of specific hearing defects in males with sex chromosome anomalies which gives further support to the hypothesis of the X chromosome having an etiological significance for hearing defects

MATERIAL AND METHODS

The case material consisted of 26 men with different sex chromosome anomalies 19 had a 47,XXY two a 46 XY/47,XXY one a 46 XX/47 XXY and four a 47 XYY chromosome constitution The age at examination varied between 19 and 58 years with a mean age of 33 years

The expenses for the present study were covered by a grant from the Swedish Medical Research Council

Table I *Audiometric analysis in 26 male patients with sex chromosome anomalies*

Case no	Initials	Chromosome constitution	Age at examination (years)	Audiometric analysis: Hearing threshold type of impairment	Audiometric analysis: Stapedius reflex threshold[a]
1	B R	47 XXY	19	—	–
2	L. H	47 XXY	20	—	–
3	R L.	48 XXXY	21	—	+
4	T R.	47 XXY	21	—	+
5	K N	47 XXY	21	—	+
6	L J	47 XXY	22	—	–
7	B R	47 XXY	22	Bil slope from 1 kHz	–
8	L H	47 XXY	25	—	–
9	H C	47 XXY	29	Left Dips 0.5–1 5 kHz Max 1 kHz 20 dB Right —	+
10	Å F	47 XXY	30	—	–
11	E. O	47 XXY	31	—	+
12	L. S	46 XY/47 XXY	33	—	–
13	H B	47 XXY	33	—	+
14	P S	47 XXY	36	—	+
15	K-O L	47 XXY	42	—	+
16	P E. H	46 XY/47 XXY	42	Bil sym dip 2–8 kHz	–
17	P H	46 XX/47 XXY	44	—	–
18	J H	47 XXY	46	—	–
19	O J	47 XXY	46	Bil sym dip 2 2–7 kHz Max 3 kHz – 55 dB	–
20	E. M	47 XXY	47	Bil slope from 1 5 kHz	–
21	R D	47,XXY	50	Bil slope from 1 kHz	–
22	R S	46 XY/47 XXY	58	—	–
23	U I	47 XYY	20	—	–
24	L. S	47 XYY	30	—	–
25	K-O S	47 XYY	27	—	–
26	B L.	47 XYY	44	—	–

[a] – = normal + = pathologically high

The audiometric tests were performed by trained operators under satisfactory room conditions The technical performance and calibration of the equipment were checked as conforming to IEC 177 (1965) and ISO R 389 (1964)

The hearing threshold was recorded by Bekesy audiometry This method which provides a detailed pattern of the hearing threshold over the whole frequency range can disclose even minor hearing defects too small to be safely recorded in ordinary octave audiometry

The patients were also submitted to a stapedius reflex test using the technique described by Klockhoff & Anderson (1959) Klockhoff (1961) and Anderson (1969) In addition to its usefulness as an objective test of the ear s sensory function at high sound stimulus levels this test is sensitive enough to exclude any interference from disorders of the middle ear (Klockhoff 1961) The procedure consequently ensures that only the sensory neural capacity of the hearing organ is tested

In classifying hearing defects one must consider that the hearing threshold may be affected by such exogenous factors as noise and certain drugs and that there may be a decline in hearing sensitivity with increasing age In the present work reference was made to the mean values and to the general shape of the hearing thresholds of the corresponding age and sex groups of the Wisconsin Hearing Survey (Glorig et al 1957) Frequency restricted defects in the hearing threshold dips were classified according to Anderson & Wedenberg (1968)

RESULTS

No conductive or mixed hearing impairments were found in the 26 patients studied while

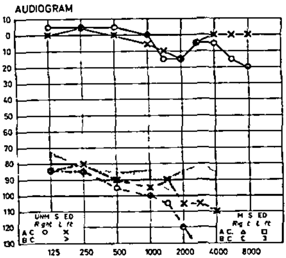


Fig 1 Hearing and reflex thresholds in a characteristic case of Klinefelter s syndrom Shaded area Normal range of stapedius reflex thresholds (Case 3)

had a significant elevation of the hearing threshold of a pure sensory neural type (Table I) Five of these 6 subjects (19 %) had hearing threshold defects of apparently endogenous origin whereas an exogenous cause could not be ruled out in 1 case (no 19) Four of them (nos 7 16 20 and 21) had a hearing impairment of the high frequency type with a slope starting below 2 kHz and one (no 9) had a significant dip in the middle frequency range (dip centre 1 kHz) Seven other patients displayed clear dips which however were too small to fulfil the criterion with respect to amplitude (cf Fig 1)

The reflex thresholds were classified according to Anderson & Wedenberg (1968) Nine of the patients (35) had pathologically high reflex thresholds Another 5 patients had suspiciously high reflex threshold which however did not satisfy the pathologic criteria A characteristic hearing and reflex threshold in one of the patients is shown in Fig 1

DISCUSSION

While the material is too small to permit definitive conclusions the number of hearing threshold impairments of probably endogenous origin seems unusually high (19 ′) Only 1 case fulfilled the criterion of a frequency restricted hearing threshold defect of a certain amplitude a dip but a further 7 cases demonstrated clearly identifiable minor changes This finding is of interest since this specific type of hearing threshold pattern is also recorded in nearly half of the cases with another sex chromosome anomaly Turner s syndrome Furthermore a similar threshold configuration is found in presumptive carriers of autosomal recessive genes for deafness (Anderson & Wedenberg 1968)

The most pronounced feature in the present material was however pathologically high stapedius reflex thresholds which were found in nine cases (35 %) Such changes are only found in about 3 % in control series (Anderson & Wedenberg 1968) Only a few non endogenous causes of abnormally elevated reflex thresholds are known (Anderson 1969) but none of them affords an acceptable explanation in the present cases On the other hand our observations are highly consistent with the fact that abnormally high stapedius reflex thresholds may occur in spite of a normal hearing threshold Also this finding in males with an extra X chromosome is similar to that in the presumptive carriers of autosomal recessive genes for deafness The present series of four XYY males does not allow any conclusion with regard to the significance of the Y chromosome

A comparison between the results of the hearing examinations in the present material of male patients with sex chromosome anomalies and the earlier studied group of Turner patients shows that (i) hearing threshold dips occur more seldom and are less pronounced in the male group and (ii) in contrast to the female group pathologically high stapedius reflex thresholds are recorded only in the male group The hearing threshold and reflex threshold patterns in the group of male patients resemble more closely those observed in hereditary hearing defects of the recessive type than of the Turner type Thus abnormalities of the X chromosome seem to be of importance for the origin of certain hearing defects The mechanism however is still unknown

ZUSAMMENFASSUNG

An 26 männli hen Patienten mit unterschiedlichen Typen von Geschlechtschromosomanomalien wurden Gehöruntersuchungeen durchgeführt. Die Patien en waren in klinischer und zytogenetischer Hinsicht genauestens un ersucht und zeigten zumeist den Typ 47,XXY d.h. Klinefelter s Syndrom. Die Zahl der Fälle mit Hörverlusten wahrscheinli h endogen n Ursprunges war in dieser Gruppe 19 • d.h. ungewohnlich hoch. Mehrere der Fälle zeigten zudem schmale aber deutli h ausgeprägte Dips im mittleren Frequenzbereich. Dieser Befund ist von besonderem In eresse da d r vorliegende spezielle Hörschwell ntyp auch in nahezu d r Halfte der Fälle einer andern Geschlechtschromosomanomalie nämlich bei Turner s Syndrom, festgestellt werden konnte Beachtenswert ist weiterhin, dass eine ähnliche Horschwellenkonfiguration auch bei prasumtiven Träcern von autosomalen rezessiven Genen, die Schwerhorigkeit zur Folge haben konnen, gefunden word n ist. *Im Unterschied zu den Patienten* mit Turners Syndrom zeigte das vorliegende Material jedoch zu 35 • eine pathologisch erhohte Stapedusrefl xschwelle

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TOXIC EFFECTS OF STREPTOMYCIN AND KANAMYCIN UPON THE SENSORY EPITHELIUM OF THE CRISTA AMPULLARIS

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Abstract The pathological patterns of the sensory epithelium of the crista ampullaris of a guinea pig were studied after streptomycin and kanamycin administration The silver reaction method and succinic dehydrogenase staining were applied for the entire surfaces of all specimens Differences in sensitivity to antibiotics were confirmed in the sensory cells of the central and the peripheral regions of the crista (Lindeman 1969 *a*)

The sensory cells of the vestibular organ are known to be so sensitive that after a long term or high-dose administration of streptomycin or other aminoglycosidic antibiotics they are damaged Although the cytoarchitecture of the sensory epithelium on the crista ampullaris is not as regular as in the organ of Corti one can easily compare its pathological pattern with that of a normal one In recent years Lindeman (1969 *a*) studied the effects of streptomycin and kanamycin upon the pattern of the sensory cell loss with Engstrom s surface specimen technique (Engstrom et al 1966) using the phase-contrast and light microscope In the present study the same subject demonstrating the overall view of the sensory epithelium was discussed In these experiments the silver reaction method (Watanuki 1968) and succinic dehydrogenase staining (Barka & Anderson 1963) were used Both methods which have already been applied in studies of the surface areas of the vestibular organ (Watanuki & Meyer zum Gottesberge 1971) have also been used in the present study

MATERIAL AND METHODS

Forty eight young pigmented guinea pigs each weighing about 300 g were divided into four groups In the first two groups streptomycin sulfate or kanamycin sulfate (250 mg/kg) was injected intraperitoneally every other day for a period of 8 weeks while the second two groups were injected streptomycin or kanamycin sulfate (about 0 6 ml of the same concentration) into the middle ear cavity via the tympanic membrane under barbiturate anesthesia three times with the interval of 1 week All animals were sacrificed approximately 2 weeks after the last dose The temporal bones were then removed the stapes was extracted and the bony walls around it as well as the three ampullae were chopped away The membranous walls of the saccule utricle and three ampullae were perforated and then the temporal bone was immersed in a 0 2% $AgNO_3$ solution at a temperature of 2 to 5 C for 2 min The temporal bone was then washed in distilled water for a few seconds and fixed in a 10 formalin solution under fluorescent or daylight for over 24 hours prior to use For the succinic dehydrogenase staining the exposed temporal bones were incubated in the following solutions for 30 min at a temperature of 37 C

Sodium succinate 0 06 M 1 0 ml
Nitro BT 0 2 2 5 ml

Phosphate buffer 0 2 M (pH 7 4) 1 0 ml
Ringer solution 0 5 ml

After a 24 hour fixation in 10% formalin each sensory epithelium was then peeled out with a sharp needle Under a cover glass the specimen was covered with a drop of glycerin For observation a light microscope was used

RESULTS

The sensory cells of all animal groups were more or less damaged and some of them dis appeared being replaced by the supporting cells In this study streptomycin proved to be more toxic than kanamycin The degeneration of the sensory epithelium was most pronounced in the group of animals which were injected strep- tomycin directly into the middle ear while the sensory epithelia of those which were intra peritoneally injected kanamycin showed the least degeneration As can be seen on Figs 2 and 5 the first degenerative changes were caused in the central part of the sensory epithe lium of the crista Most of the larger sensory cells in the central part collapsed but a small umber of the smaller cells remained still un volved In a severely damaged sensory epithe lium only a few scattered sensory cells were found in the peripheral regions of the sensory epithelium (Fig 3) Despite the extensive dam age done to the sensory cells the supporting cells remained uninjured replacing the collapsed sensory cells Finally the sensory epithelium be came flattened and no sensory cell was found on the sensory epithelium which now consists of only the hypertrophied supporting cells The sensory cells of type I were more vulnerable than those of type II in both central and peripheral regions The sensory cells of type II in the central region however were more sensi tive than those of type I in the peripheral re gion. The sensory cells of type I innervated by common nerve chalices were not found in any specimens examined in this study indicating that they are most vulnerable in antibiotic oto- toxicosis

COMMENTS

The toxicity of the aminoglycosidic antibiotics on the vestibular sensory cells has been well known (Streptomycin Hawkins 1947 Hawkins & Lurie 1952 Winston et al 1948 Causse 1949 Ruedi et al 1952 Ruedi, 1951 Chris tensen et al 1951, Berg 1951 Lurie 1955 Schuknecht 1957 McGee & Olszewski 1962 Wersall & Hawkins 1962 Duvall & Wersall 1964 Spoendlin 1966 Igarashi et al 1966 Nagaba 1968 Lange 1968 and others Kana mycin Farkashidy et al 1963 Lindeman 1969 *a* Viomycin Kanda & Igarashi, 1969) Many authors reported of a more severe dam age done to the summit or apex of the crista than to the slopes of the sensory epithelium which they tried to prove by observing the con ventional cross sections of the crista Lindeman (1969 *a*) applied the surface specimen technique in normal and pathological studies of the sen sory epithelia of vestibular organs and found that both lateral parts of the top of the crista adjacent to the planum semilunatum are also less sensitive to toxicity just like the slopes of it. The present examinations confirmed Linde man s findings by the observation of the surface specimens stained by the two different staining methods It should be emphasized that in addi tion to the studies it is also inevitable to study the longitudinal sections of the crista ampul- laris The degree of sensitivity of the vestibular sensory cells to ototoxic antibiotics is reported to be different between the type I and the type II sensory cells Wersall & Hawkins (1962) main- tained that the type I cells were more vulner able to streptomycin than the type II cells in both the crista and the macula utriculi of th cat also Spoendlin (1966) demonstrated that this is true of the macula utriculi of the same species and Lindeman (1969 *a*) of all the ves tibular organs of the guinea pig In viomycin ototoxicosis Kanda & Igarashi (1969) found the same tendency in both the crista and th macula utriculi of the cat as well as the squirrel monkey Duvall & Wersall (1964) however found that degenerative changes of the sensory



Fig. 1. Overall view of the surface specimen of the crista posterior in a normal animal after silver reaction. The sensory epithelium as well as the supporting epithelium are clearly demonstrated. × 150.

epithelium may take place all over the sensory areas of both type I and type II sensory cells in animals treated with streptomycin. The present examinations revealed that the type I cells of the crista of a guinea pig were more vulnerable than the type II cells in the central region; however, the type II cells in the central region were generally more vulnerable than the type I cells in the peripheral region and the type I cells were in turn more sensitive than the type

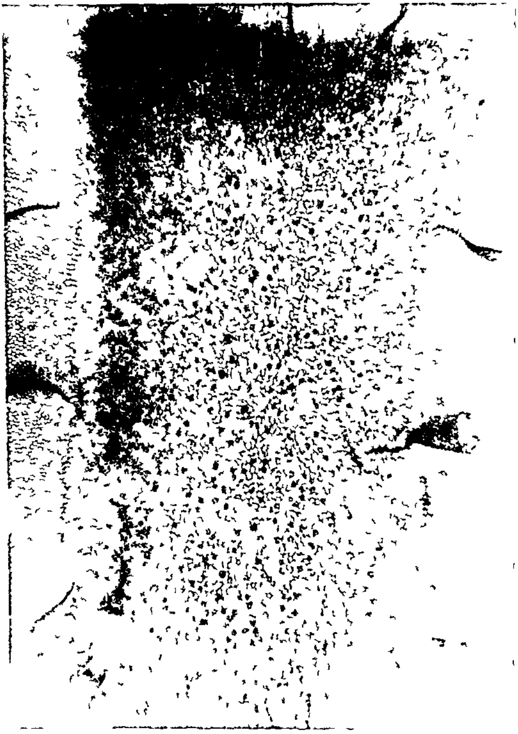


Fig 2 Overall view of the crista posterior moderately damaged by streptomycin after silver reaction In the central part most of the sensory cells were injured while in the peripheral part they remained nearly undamaged × 150



Fig. 3. Overall view of the crista anterior severely damaged by streptomycin after silver reaction. In the central part almost all sensory cells have collapsed. In the peripheral part, however, a small number of sensory cells can still be found. × 150.

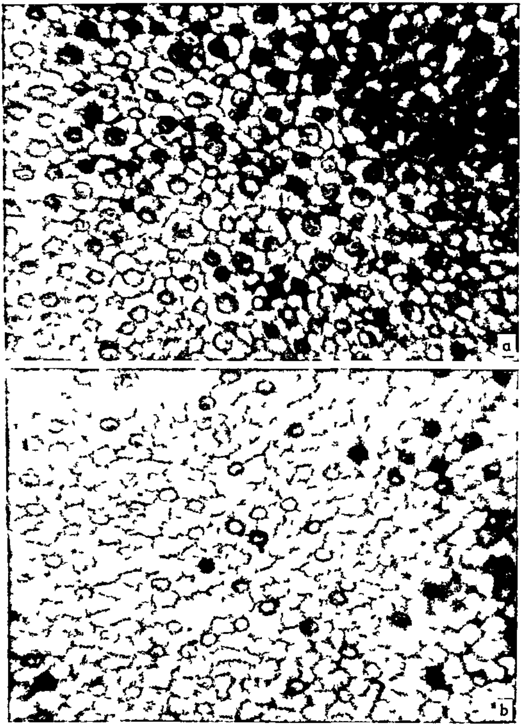
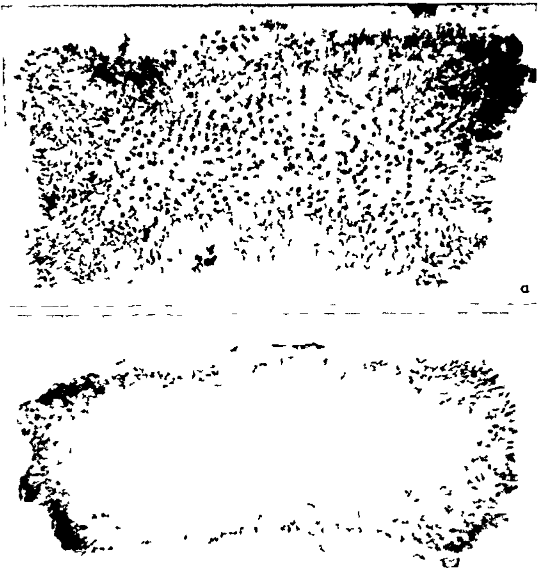


Fig 4 (*a*) Surface structure of the sensory epithelium in a normal animal. The sensory cells are joined with the supporting cells which form the reticular lamina at the surface of the epithelium × 1 200 (*b*) Surface structure of the sensory epithelium in a streptomycin treated animal after silver reaction The sensory cells are rarely found The supporting cells were hypertrophied and replaced the sensory cells × 1 200

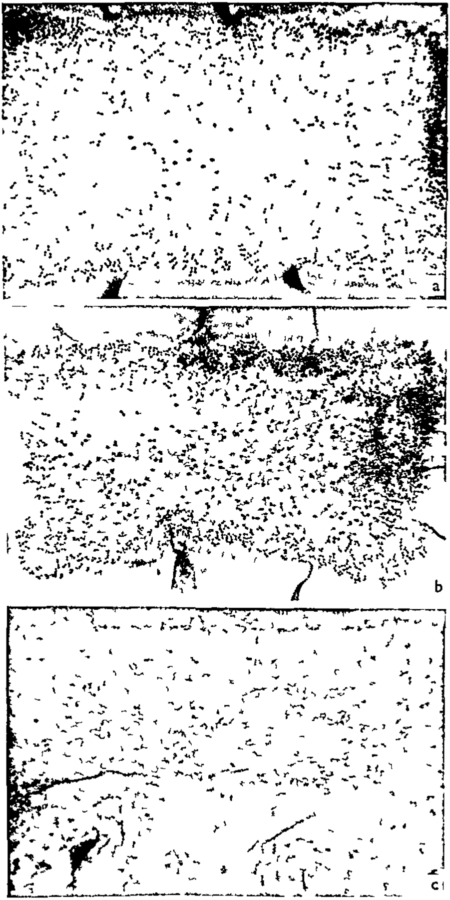


a

b

Fig 5 (*a*) Overall view of the surface specimen of the crista lateralis in a normal animal after succinic dehydrogenase staining Only the sensory cells are distinctly stained. 130 (*b*) Overall view of the surface specimen of the crista posterior in a streptomycin treated animal Sensory cell loss in the central part is clearly visible × 130

II cells in the peripheral region It has been discussed that the type I cells which are phylogenetically younger and more highly differentiated than the type II cells are more sensitive to the antimetabolic action of antibiotics (Wersall & Hawkins 1962 Spoendlin 1966) Lindeman (1969 *a*) emphasized however that the degenerative pattern after exposure to ototoxic agents is dependent upon the specificity of the agent In any event the morphological characteristics of both the type I and type II sensory cells should be emphasized by interpretation of this difference with respect to their sensitivity The biggest part of the type I sensory cell body is isolated from the neighbouring supporting cells with the nerve chalice interposed only the


a
b
c

small neck part contacting with the supporting cells This indicates that the supply of oxygen and nutrients from the supporting cells is less established in the type I cells than in the type II cells Winther (1969) however detected that the type II cells were easier damaged by X ray irradiation than the type I cells Yet this question still remains unsolved In the present study no common nerve chalices were detected not even in the least damaged specimen of the crista It is likely that the type I cells which are innervated by common nerve chalices are the most vulnerable in ototoxicosis caused by streptomycin and kanamycin

ZUSAMMENFASSUNG

Die pathologischen Veranderungen des Sinnesepithels der Crista ampullaris beim Meerschweinchen wurden nach Gabe von Streptomycin und Kanamycin untersucht Zu diesem Zweck wurden an den Hautchen praparaten die Silberreaktion und der Succinodehydrogenasenachweis angewandt Der Sensibilitatsunterschied der Antibiotikaototoxikose zwischen dem zentralen und peripheren Bereich der Crista konnte bestatiet werden (Lindeman 1969 *a*)

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THE ACUTE EFFECT OF ULTRASONIC IRRADIATION UPON AMPULLAR SENSORY EPITHELIA OF THE GUINEA PIG

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Abstract Acute experimentation with ultrasonic irradiation of the guinea pig labyrinth indicates that the physical effect of the ultrasonic irradiation such as agitation cavitation and streaming are the prime factors in explaining damage to the sensory cells with ruptured cytoplasmic membranes disappearance of ribosomes and mitochondria and formation of intracytoplasmic vacuoles which were found even one or two minutes after treatment The basic pattern of degeneration is the same whether the perilymphatic space is open or not The thinner the bony shell remaining the shorter the exposure which can be used to achieve the same degree of destruction thus minimizing heat transfer to the surrounding tissues In the present acute experiments no cochlear damage was observed

During the last decade the Meniere s disease has been treated with many methods such as labyrinthectomy with ototoxic antibiotics or by surgical techniques various operations of the endolymphatic sac medical treatment and labyrinthine destruction without opening the bony capsule of the labyrinth by using either ultrasound or cryo-surgery Many surgeons believe that there is a greater possibility of saving the cochlear function of patients when using the latter techniques For that purpose ultrasonic irradiation has during the last decade been extensively used by Angel James et al (1960 1969 *a b*) Altmann & Waltner (1961) Arslan (1953 1966) and Sjoberg with coworkers (1963) among others

Although several authors (Angell James et al 1964 Sjoberg et al 1963 Stahle 1964 Dominok & Preibisch Effenberger 1965 among others) have reported histological evidence of degeneration of the labyrinth after treatment, submicroscopical studies have not been performed to clarify the possible process of cytological destruction

The following report presents the results of some acute experiments with ultrasonic treatment followed by an immediate sacrifice of the animals Investigations of the ampullar epithelia were performed with the electron microscope in order to shed some light on the initial morphological changes in the ampullar cristae after treatment

MATERIAL AND METHODS

Twelve young healthy guinea pigs were used All operations were performed under general anaesthesia A postauricular approach was adopted to open the bony capsule over the ampullae of the superior and lateral semicircular canals The bony covering of the labyrinth was thinned down to a blue line to expose the membranous labyrinth In some animals the bone was completely removed The ultrasonic probe was applied for times ranging from 2 5 to 15 min at an intensity of 22 W/cm The probe area was 0 03 cm and a frequency

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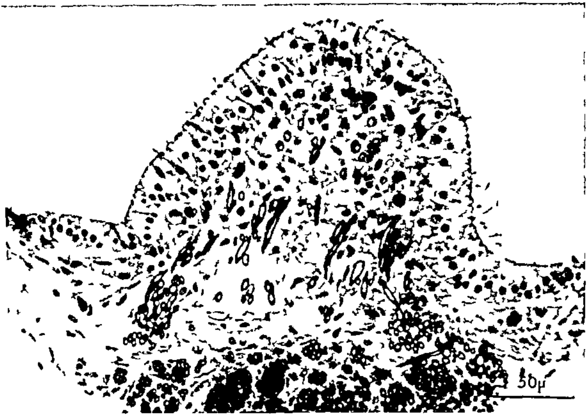


Fig 1 Ultrasound 10 min 22 W/cm The sensory epithelial lining appears superficially normal Nevertheless some vacuoles are found in the hair cell cytoplasm Congestion of the blood vessels in the subepithelial connective tissue is also noticeable

of about 3 MHz was used with a Bristol Elliot machine (cf Angell James 1963) under continuous irrigation with saline The temperature of this coolant varied from +28° to +34 C at the tip of the probe under surgery After the treatment the temporal bones were immediately removed and the specimens were fixed and embedded according to routine procedure either for the celloidin technique or for electron microscopy using the epoxy resin Epon

Findings after various levels of ultrasonic irradiation

When the bone was completely removed there were only minimal differences between the results after 2 5 and 15 min of irradiation In these specimens the whole connective tissue stroma of the ampullar cristae appeared vacuolarized and slightly shrunk while the sensory cells and supporting cells were separated from the underlying stroma by rupture at the bottom of the supporting cells or the basement membrane The space between these two layers seemed to be filled mostly with fluid and only partly with amorphous material By light microscopy it was not possible to investigate the detailed changes in the epithelial lining itself Nevertheless the mentioned changes in gross morphology could readily be observed When a blue line that is a thin shell of bone was left intact covering the perilymphatic space however a more gradual degeneration was found progressing according to the length of treatment The present work has been carried out to describe the changes in the sensory epithelium after the same intensities of irradiation but with consecutively lengthened irradiation periods (Figs 1 2)



Fig 2 Ultrasound 15 min 22 W cm A typical finding at high energy levels is a rupture of the epithelial stroma pronounced at the top of the ampullae The connective tissue stroma appears somewhat shrunk when compared with the bulging epithelial lining Note that the fluid surface of the sensory cells still appears remarkably intact

1½ minutes After this brief treatment the major finding was a sludging of the red blood corpuscles of the capillaries in the connective tissue stroma of the ampullar cristae This was observed with light microscopy however no cytological changes in the epithelium itself could be observed With electron microscopy on the other hand some sensory cells already showed a slight vacuolization of the cytoplasm and some nuclear condensation of chromatin was noticeable

5 minutes At this level of irradiation a more extensive vacuolization in both the sensory cells and the supporting cells were observed especially at the top of the cristae No difference in the pathology of type I and type II cells could be ascertained The most characteristic finding was disintegration of the mitochondria (Fig 3) disappearance of the ribosomes and other intracytoplasmic organelles The nuclei were pyknotic and sometimes the nuclear membrane had deformed or ruptured (Fig 4) The same findings could be observed in the supporting cells and nerve chalice surrounding the type I cells and in the nerve endings The sensory hairs were astonishingly well preserved The cuticle and junctions between hair cells and supporting cells appeared intact (Fig 5) Some ruptures appeared at the bottom of hair cells and supporting cells especially at the top of the crista These changes were less prominent along the sides of the crista towards the transitional cell zone Some degeneration of the myelinated nerves was found in the subepithelial stroma

10 minutes Not only was the ampullar crest degenerated but the separation of the sensory



Fig 3 The first changes observed on the application of ultrasonic energy are rupture of the mitochondrial cristae with formation of clear zones in the mitochondria (*Mi*) and formation of clear spots in the cytoplasm of the sensory cells with pronounced protrusion of the apical cytoplasm into the endolymph (*arrow*) The cuticle (*Cu*) with its sensory hairs (*SH*) appears still intact The cytoplasm of the surrounding nerve chalice (*NCh*) also exhibits some signs of degeneration with formation of clear spots



Fig. 4. In a more advanced state of degeneration the cytoplasm (*Cy*) appears devoid of ribosomes. Vacuoles (*Va*) are formed and in the nuclei (*Nu*) condensation of chromatin as well as clear spots (*arrows*) are found. The nerve chalices (*NCh*) exhibit similar signs of degeneration as also do the secretory cells (*ScC*).



Fig 5 At high energy levels the cytoplasm becomes filled with vacuoles (*arrow*) and appears almost watery in appearance with only few remnants of its organelles The fluid surface with the sensory hairs and cuticular part of the sensory cell is still much less changed The nuclei (*Nu*) at this stage exhibits severe pyknotic changes

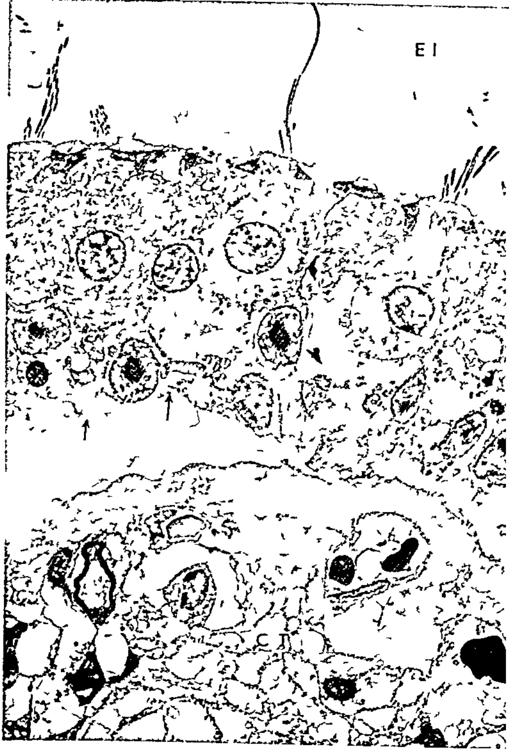


Fig 6 Rupture of the epithelial lining with formation of fluid filled space between the connective tissue (*CT*) and the epithelial lining (*arrows*) is observed at prolonged treatment or very high energy levels This rupture mostly occurs inside the supporting cells with the basement membrane still adhe to the subepithelial connective tissue Although sensory cells are severely damaged they can still recognized Endolymphatic surface (*EI*)

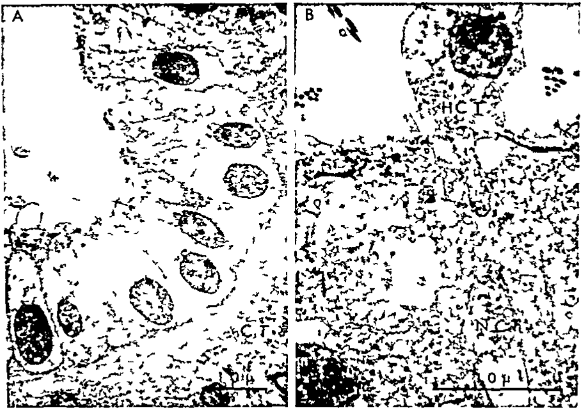


Fig 7 (*A*) The side walls of the cristae with the transitional cell layer are less affected than the ampullar crest Degeneration of the transitional cells with formation of vacuoles and watery appearing cytoplasm is observed However the cells are very rarely disconnected from the subepithelial connective tissue (*CT*) (*B*) In an advanced state of degeneration the sensory cells are often found being expelled into the endolymph as this hair cell type I (*HCI*) The nerve chalice (*NCh*) appears compressed by the surrounding secretory cells

epithelium from the basement membrane could be observed down to the transitional cell layer at the sides of the ampullae (Fig 6) In the fluid surface of the sensory cells tears now appeared and some cells were ruptured and cytoplasm was expelled into the endolymph (Fig 7) The hairs seemed to have disappeared from some cells Edema of the nerve chalices was constantly present and the contracts between the cellular walls were irregular in appearance The transitional cells also exhibited a vacuolar cytoplasm devoid of cellular organelles rupture of the nuclei and a bursting fluid surface They were however very rarely separated at their basal end from the connective tissue beneath (Fig 7)

15 minutes Most of the sensory epithelial cells were completely degenerated and rupture of the cytoplasmic membrane was a constant feature The degenerative findings could be followed towards the ampullar floor and to the planum semilunatum No particular additional finding was noticed except for an exacerbation of the above described changes

Cochlea

With the technique used in this series of experiments no immediate cochlear damage was observed in the basal turn even in the 15 min experiment The top of the sensory hair cells were intact with all the sensory hairs remaining The apical cytoplasm exhibited the

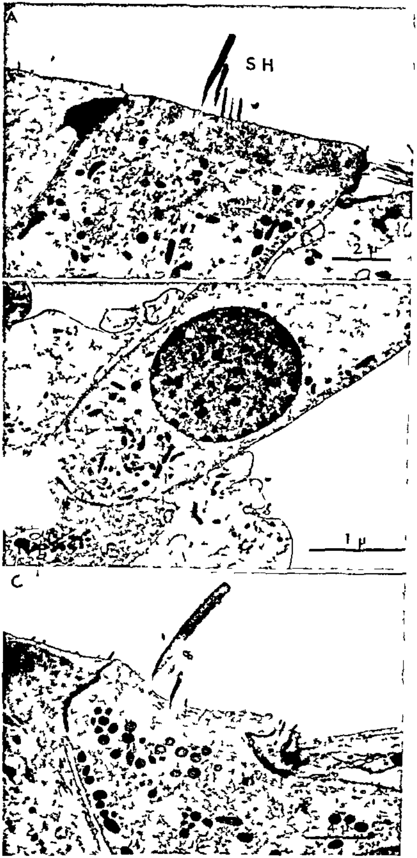


Fig 8 The cochleae of this acute series of animals were morphologically normal even with an exposure of 15 min of 22 W/cm as demonstrated in sections from a basal turn (*A*) Normally appearing sensory hairs (*SH*) and endolymphatic surface of an outer hair cell (*B*) Nerve ending (*NE*) region (*C*) Top of inner hair cell

cellular organelles and no rupture of the mitochondria was found either in the outer or in the inner hair cells The nerve endings at the bottom of the sensory cells were equally unaffected as were the cells of Deiters and Hensen (Fig 8)

DISCUSSION

Angell James (1963 1969) and Sjoberg et al (1963) have in an excellent way described the physical characteristics of the ultrasonic beam as well as its possible ways of affecting the labyrinth The possible biophysical effects are summarized as follows

Agitation which may cause ruptures of the cells

Streaming with formation of bubbles in the cellular cytoplasm with blocking of the metabolic systems

Cavitation with formation of gas bubbles in the cells

Rise of temperature

Chemical changes such as oxidation and degeneration of large molecules and alterations of pH due to release of free radicals

Increased permeability of cell membrane at low intensities and rupture at high intensities

Increased biochemical activity of enzymes in cells at low intensities and destruction at high

These biological effects on the sensory cells as summarized above according to Angell James (1969 *b*) are of course impossible to analyse completely with pure morphological methods However the presently described findings in this paper could give some informations about the actual changes in the ampullar sensory cells

The main discussion of previous authors has been whether the primary effect on the sensory cell is due to heat or to the other physical effects by the ultrasonic beam on the submicro-cytoplasmic components of the cell It been shown that a substantional rise in temperature could be found at the transition between media of different densities such as between bone and the fluids of the inner ear as well as between the coupling medium between the transducer and bone especially when the tip of the ultrasonic probe was hot as in the Italian Federici apparatus as introduced by Arslan in 1953 Due to the heat transfer in the tip and the large surface area of that equipment cases of facial nerve trauma were reported in the early fifties by Altmann & Waltner (1961) This led Angell James (1963) to introduce the Bristol Elliot Machine which works with a free flow of saline through the probe over the irradiated areas thus minimizing the risk of heat transfer to the temporal bone and also acting at the same time as an efficient coupling medium to the labyrinth Another approach was made by the Sjoberg group in Sweden with the Ultrapoint Machine (Sjoberg et al 1963) with closed treatment heads however cooled by circulating distilled water conducting the ultrasound from the crystal to the tip and also cooling it.

In our investigation a Bristol Elliot Machine was used with intensities up to 22 W/cm and irradiation time up to 15 min It appeared that complete removal of the bone with introduction of the tip into the perilymphatic space gave a very pronounced effect already at low dosages At the intensities used an almost complete destruction of the lateral and superior ampullae was made without possibility of describing any subtle morphological changes When however a thin bony lamella as a blue line was left over the perilymphatic space the changes observed related well with the amount of energy applied However it was found that some variations occurred in different animals possibly due to difficulties in achieving exactly the same bony removal angulation of the probe tip and contact with the bony surface of the labyrinth From various cristae we have been able to analyse a pattern of degeneration related to increasing irradiation time

The first changes observed were intracytoplasmic with appearance of clear spots in the

cytoplasm and later rupture of the mitochondria and formations of intracytoplasmic vesicles Formation of lysosomes was not observed There was instead a subsequent complete disintegration of the cytoplasmic contents including ribosomes The nuclei were at first unaffected but later a definite pyknosis with irregular distribution of the chromatin was present At high intensities even a clearing of the nucleoplasm and rupture of the nuclear membrane occurred

At high energy levels these changes progressed with rupture of the sensory cell surface rupture of the basement membrane with formation of fluid between the epithelial lining and connective tissue stroma It is tempting to believe that we have here pure agitation as the main effect of the ultrasonic irradiation equally affecting both sensory cells and supporting cells

Facial nerve paralysis was found in two animals in which a thick bony shell remained over the perilymphatic space with a possible increase of heat transfer at that surface In these animals exudation of fibrinous aterial was observed in the ampullae

Cochlear changes were not found in the present series of animals However it is possible that in the long term survival experiments which will be reported later we will find some degeneration since we know from clinical experience that this is sometimes the case

From our experiments it seems most likely that the immediate effect of ultrasound on the sensory cell is due to the physical effect of the vibration on the cell walls and cellular organelles with the absorption of the energy in the tissues especially at all interfaces between cytoplasm and its organelles where a change in impedance (density) is present

Actual transfer of heat from the probe tip seems not to be the likely cause of damage With a frequence of 3 MHz as used the half intensity power distance is several metres in water and just about 1 mm in the temporal bone (Angell James 1969) This is clearly reflected by the fact that the thinner the remaining bone the shorter the period of application which could be used to give the same degree of degeneration yet minimizing the risk of heat transfer to the neighbouring facial nerve

ZUSAMMENFASSUNG

Akute Experimente mit Ultraschalldestruktion des Labyrinths vom Meerschweinchen zeigen dass der physikalische Effekt der Ultraschallbestrahlung wie Agitation und Cavitation am besten das Vorkommen von Sinneszellendestruktion mit rupturierter Cytoplasmamembran verschwindenen Ribosomen und Mitochondrien sowie die Bildung von intracytoplasmatischen Vacuolen erklart Diese Effekte liegen schon zwei Minuten nach der Behandlung vor Das Degenerationsmuster bleibt das Gleiche ob man den perilymfatischen Raum offnet oder nicht Je dunner die Knochenschicht ist die man uber dem Labyrinth belasst desto kurzere Ultraschallexposition kann angewendet werden um den gleichen Grad der Destruktion zu erreichen welches gleichzeitig ein kleineres Risiko fur Warmedestruktion des umgebenden Gewebes z B des Fazialisnerven bedeutet In der vorliegenden Arbeit kamen keine cochlearen Schaden vor

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BIOCHEMICAL EFFECTS OF THIETHYLPERAZINE (TORECAN®) ON THE VESTIBULAR SYSTEM OF RABBITS

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(Received December 3 1970)

Abstract Albino rabbits were subjected to unilateral vestibular neurotomy and sacrificed 7 days later One group received intravenous injections with thiethylperazine (Torecan) 6 5 mg in 1 ml solution twice daily Controls were injected with Ringers solution Succinoxidase activity was determined in Deiters giant cells and RNA content in Purkinje cells from hemisphere lobulus III Incorporation of ^{3}H leucine into protein from cerebral cortex lateral vestibular nuclei and cerebellar nodulus and hemisphere lobulus III was determined in neurotomized rabbits and in rabbits given thiethylperazine Neurotomy caused pronounced vestibular disturbances and the animals had higher succinoxidase activity on the operated than on the non operated side Rabbits treated with thiethylperazine showed minor signs of vestibular disturbance and the Deiters cell succinoxidase activity was similar on both sides as well as the Purkinje cell RNA content 1 week after the operation The ^{3}H leucine incorporation into protein from cerebellum was higher on the operated side whereas no side differences were observed in the cerebral cortex and lateral vestibular nuclei

In a series of studies disturbances in the rabbit vestibular system were caused by inducing changes in the impulse flow impinging upon central vestibular parts such as the lateral vestibular nucleus and the cerebellum This was performed by unilateral division of the vestibular nerve (Blomstrand et al 1966 *a* Jarlstedt 1966 Hallen et al 1967) by unilateral caloric stimulation (Blomstrand et al 1966 *b* Jarlstedt 1966) cerebellectomy (Blomstrand et al 1968 *a* *b* 1970 *a*) or hemicerebellectomy (Blomstrand et al 1970 *b* *c*) In these earlier studies we investigated the RNA content and the succinoxidase activity in single isolated giant neurons (Deiters cells) from the lateral vestibular nucleus and the RNA content in single Purkinje cells from those parts of the cerebellum which have vestibular connections Protein synthesis in these areas has also been measured (Blomstrand et al 1970 *a*) We have succeeded in obtaining cytochemical correlates to altered functions in the vestibular apparatus and investigated the central compensation after vestibular neurotomy Unilateral vestibular neurotomy (Blomstrand et al 1966 *a*) increased the succinoxidase activity in Deiters cells on the operated side This was marked 6 days after neurotomy and reached its maximum after 15 days One month after the operations the succinoxidase activity of the Deiters cells did not differ from control values The RNA content in the Deiters neurons did not change after vestibular neurotomy whereas the Purkinje cell RNA content decreased on the operated side and was not restored to normal after 30 days The cytochemical changes in the lateral vestibular nucleus were dependent on intact vestibular parts of the cerebellum since a lesion of the cerebellar nodulus in direct sequence with the unilateral neurotomy (Hal

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len et al 1967) abolished the earlier observed increase in succinoxidase activity in D iters cells We have used this cytochemical system as a model for the study of drug action on central vestibular function The effect of thi ethylperazine was investigated in control ani mals and in animals subjected to vestibular neurotomy since a number of clinical studies have shown that thiethylperazine is effective in the treatment of nausea and vertigo (for review see Rubensohn 1970) The drug accumulates particularly in cerebellar areas (Sparks et al 1962 Jaramillo & Guth 1963)

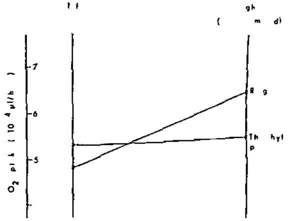


Fig 1 Succinoxidase activity in Deiters cells from rabbits subjected to unilateral neurotomy (right side) 1 week before sacrifice Influence of postoperative intravenous injections of thiethylperazine (6 5 mg per day) compared with Ringer s solution The activity is expressed as O consumption (μl O $\times 10^{-4}$/hour)

MATERIAL AND METHODS

Albino rabbits weighing 1 5–1 8 kg were used in the present investigation Vestibular neurotomy was performed unilaterally as reported in an earlier investigation (Blomstrand et al 1966 *a*)

Cytochemical investigations

One group of unilaterally neurotomized ani mals were injected intravenously with 6 5 mg thiethylperazine in 1 ml of solution (Torecan® Sandoz AG) twice a day postoperatively Another group of neurotomized animals re ceived daily intravenous injections of Ringer s solution (controls) instead of thiethylperazine All animals were killed by air emboli 7 days after operation The brain stem was quickly removed and Deiters giant nerve cells were dissected out from the fresh tissue by free hand from the lateral vestibular nucleus and carefully separated from the surrounding neuro pil under a stereo microscope (Hydén & Pigon 1960) The succinoxidase activity of single nerve cells was assayed manometrically by the micro diver method (Zeuthen 1953) The in cubation medium had the following composi tion (final concentrations) Na HPO_4–KH PO_4 buffer pH 7 4 37 5 mM $AlCl_3$ 0 5 mM MgCl 0 5 mM Na succinate 25 mM cyto-chrome *c* 8 6 $\times$ 10 M In a limited series of experiments the RNA content of isolated Dei ters cells from the lateral vestibular nucleus and Purkinje cells from the cerebellar hemi-sphere lobulus III was analysed on both sides 1 week after unilateral vestibular neurotomy (Blomstrand et al 1966 *a*)

Incorporation of ³H leucine into protein

The influence of unilateral vestibular neuro-tomy on ^{3}H leucine incorporation into protein was investigated in various brain areas (cortex cerebri lateral vestibular nuclei and cerebellar nodulus and hemisphere lobulus III) 7 and 14 days after the neurotomy In another series the *effect of thiethylperazine on intact animals* was investigated by intravenous injection of the drug and the ^{3}H leucine incorporation was determined in the above mentioned areas Group I received 6 5 mg/day for 2 days be fore sacrifice and group II were injected with 32 5 mg/day for 7 days The brains were removed immediately after sacrificing the rab-bits by air emboli The different regions were dissected out chopped and incubated in buf fered salt media containing ^{3}H leucine (10 μCi/ ml) as described previously (Blomstrand & Hamberger 1970) The material was homo-genized in 0 32 M sucrose containing Tris HCl

pH 7 4 10 mM and unlabelled leucine 10 mM Protein was measured by the method of Lowry et al (1951)

The protein bound radioactivity after TCA washing and lipid extraction was measured in a Packard Tricarb scintillation spectrometer as described earlier (Blomstrand & Hamberger 1970)

RESULTS

Animals

Those animals subjected to right sided vestibular neurotomy showed a tendency of rotation torsion of the neck and eye deviation to the right often with a spontaneous nystagmus towards the left After 1 or 2 days they began to eat and drink The animals which received daily intravenous injections of thiethylperazine showed consistently milder vestibular disturbances Injections of low doses of thiethylperazine in normal non-operated rabbits had no noticeable effect on their behaviour After high doses (32 5 mg/day) a slight sedative effect was observed

ytochemical results

e Deiters cells of the neurotomized controls ad a higher succinoxidase activity on the right neurotomized side (Fig 1) which is in accordance with previous studies (Blomstrand et al 1966 *a*) However in rabbits subjected to neurotomy with subsequent daily injections of thiethylperazine (6 5 mg twice per day) no side differences in succinoxidase activity of the Deiters cells were recorded indicating that the earlier observed assymmetry in the vestibular system was abolished by the drug No appreciable side differences were found in the RNA content of Purkinje cells after unilateral vestibular neurotomy with thiethylperazine injections while neurotomy *per se* has been shown to result in a significantly decreased RNA content in the Purkinje cells of the operated side after 1 week (Blomstrand et al 1966 *a*)

Table I *Incorporation of ³H leucine into TCA precipitable material in various brain regions from non operated control rabbits*

Values expressed as counts/min/mg protein Mean value ± S E M

		No of exp
Cerebral cortex	5 810 ± 550	10
Lateral vestibular region	4 150 ± 550	10
Cerebellar hemisphere lobulus III	5 730 ± 840	10
Cerebellar nodulus	5 950 ± 910	10

³H leucine incorporation in various brain regions

The protein bound radioactivity incorporated by incubation of the chopped tissue in a medium with ³H leucine is shown in Table I No significant side differences with respect to specific radioactivity were obtained in the control animals Rabbits which received 6 5 mg thiethylperazine/day for 2 days did not differ appreciably from the controls with respect to leucine incorporation into different brain regions (Table II) With the higher dose (32 5 mg/day for 7 days) there was a 15–30% increase in leucine incorporation in Deiters nuc

Table II *Effect of intravenous injections of thiethylperazine on ³H leucine incorporation into TCA precipitable material from different regions of the brain*

Groups I and II as described in Materials and Methods Results expressed as ratios experimental/control animals Mean value ± S E M

	Group I	No of exp	Group II	No of exp
Cerebral cortex	1 10 ± 0 09	5	0 92 ± 0 15	4
Lateral vestibular region	1 08 ± 0 21	6	1 14 ± 0 09	4
Cerebellar hemisphere lobulus III	0 95 ± 0 09	6	1 26 ± 0 12	4
Cerebellar nodulus	0 93 ± 0 12	6	1 10 ± 0 20	4

Table III *Effect of unilateral neurotomy (right side) upon ^3H leucine incorporation into TCA precipitable material from different regions of the brain*

Results expressed as ratios (right/left) Mean value ±S E M

	7 days	No of exp	14 days	No of exp
Cerebral cortex	1 09±0 07	4	1 04±0 16	9
Lateral vestibular region	0 96±0 06	4	1 01±0 10	9
Cerebellar hemisphere lobulus III	1 08±0 10	4	1 15±0 16	9
Cerebellar nodulus	1 22±0 11	4	1 13±0 13	9

leus and in the cerebellum while the cerebral cortex was hardly affected The influence of unilateral vestibular neurotomy on leucine incorporation into different regions of the brain is shown in Table III No side differences were revealed for cerebral cortex and the lateral vestibular nucleus while a 10–20 % higher incorporation was observed for the cerebellar hemisphere and nodulus on the neurotomized side

DISCUSSION

It is apparent from the present investigation that cytochemical analyses on single cells allows a quantitative estimation of the metabolic drug response in different brain regions By unilateral vestibular neurotomy a cytochemical dysbalance in vestibular neurons between the two sides was induced Rabbits receiving daily injections by thiethylperazine from the day of operation had very similar succinoxidase activities in their Deiters neurons from the left and right sides while rabbits receiving injections of Ringer s solution showed pronounced side differences as reported earlier (Blomstrand et al 1966 *a*) In addition thiethylperazine treatment eliminated the previously observed decrease in Purkinje cell RNA content in lobulus centralis on the neurotomized side (Blomstrand et al 1966 *a*) Thiethylperazine diminished the symptoms of vestibular disturbance induced by unilateral vestibular neurotomy but had no appreciable sedative effect At a very high dose however far above the therapeutic dose used in clinical work a slight sedative effect was observed In agreement clinical observations indicate a favourable effect of thiethylperazine on vertigo nausea and other symptoms of vestibular dysbalances (Rubensohn 1970) with small sedative side effect Furthermore nystagmographic studies have demonstrated a marked decrease of nystagmic response to positional caloric and rotational stimulation in healthy persons and in patients suffering from various vestibular disorders after administration of thiethylperazine (Bertrand 1966)

By using fluorescence techniques the level of thiethylperazine has been determined in different anatomical areas of the brain It was observed that the cerebellum had a very high concentration of thiethylperazine compared with other brain areas (Sparks et al 1962 Jaramillo & Guth 1963) Our results suggest an increased rate of protein synthesis in the cerebellum while other regions were virtually unaffected Vestibular neurotomy also affected protein synthesis preferentially in the cerebellum Since this part of the brain is an important coordinator of vestibular function it is probable that thiethylperazine displays its primary effect on cerebellar structures However an exact correlation of drug concentration and site of action is not possible as yet since no specific receptor site is known for thiethylperazine

Changes in rates of protein synthesis in various regions may give some indication of the state of dysbalance in the vestibular system Since metabolic effect due to vestibular disturbances is probably mainly displayed in a selected neuronal population it is likely a

cytochemical approach is preferable This is supported by the present study since correlation was obtained between cytochemical effects and vestibular reactions when animals with vestibular dysbalance were treated with thiethylperazine

ACKNOWLEDGMENT

The skilful technical assistance by Mrs Inga Britt Christofferson and Mrs Eva Awall is gratefully acknowledged

ZUSAMMENFASSUNG

Weisse Kaninchen wurden unilateraler Vestibularis neurotomie unterzogen und 7 Tage nach der Operation getotet Eine Gruppe wurde mit 6 5 mg Thiethylperazine (Torecan) zweimal taglich intravenos injiziert Kontrollkaninchen wurden mit Ringers Losung injiziert Deiterssche Riesenzellen vom lateralen Vestibulariskern wurden mit Hinsicht auf Succinoxidaseaktivitat und Purkinjesche Zellen vom II Lobulus Hemisphare auf die Totalmenge RNA analysiert ³H Leucin Inkorporation in Proteine im cerebralen Cortex lateralen Vestibulariskern Nodulus und III Lobulus des Kleinhirns bei neurotomierten Kaninchen und Thiethylperazinebehandelten Kaninchen studiert Neurotomie mitfuhrte ausgesprochene Vestibularisstorungen und die Tiere hatten eine hohere Succinoxidaseaktivitat auf der operierten Seite als auf der nicht perierten Seite Thiethylperazinebehandelte Kaninchen zeigten weniger markierte Zeichen von Vestibularisstorungen Succinoxidaseaktivitat in den Deiterschen Riesenzellen und der RNA Gehalt in den Purkinjeschen Zellen zeigte keinen Seitenunterschied eine Woche nach der Operation Die H Leucin Inkorporation in Proteine vom Kleinhirn war hoher auf der nicht operierten Seite wahrend im cerebralen Cortex und in den lateralen Vestibulariskernen kein Seitenunterschied beobachtet wurde

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MIDDLE EAR EPITHELIUM OF SQUIRREL MONKEY

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Abstract Detailed anatomical characteristics of normal middle ear epithelium and a topographical distribution of cell types are described for the squirrel monkey The following cell types were classified by using electron microscopy 1) ciliated tall cell 2) ciliated small cell 3) non ciliated columnar or cuboidal cell 4) non ciliated thin cuboidal or flat cell Ciliated cells were found in the region of the Eustachian tube anterior mesotympanum and hypotympanum Columnar cells were found anterior to Jacobsons nerve and flat cells posterior to this nerve which served as a reference mark Such findings are important in understanding normal physiology and are of special interest in interpreting pathological inflammatory alterations in man and animals ranging from mild (acute serous otitis media) to severe (chronic serous otitis media and mastoiditis)

The squirrel monkey serves as a reliable animal in which serous otitis and other inflammatory changes of the Eustachian tube middle ear cleft and mastoid can be produced and studied Anatomical characteristics and surgical accessibility are similar to man Any consideration of pathological changes requires a thorough understanding of normal morphology

The purpose of this report is to describe detailed anatomical characteristics of normal middle ear epithelium and a topographical distribution of cell types

MATERIAL AND METHODS

I *Electron microscopic study of the mucoperiosteum*

Six healthy monkeys weighing 500 and 1 000 g and having good pinneal reflexes were used

This investigation was supported by the National Institutes of Health Public Health Service Research grant NB07623

The right and left cavities were perfused *in vivo* the right with 1 % OsO_4 buffered with 0 1 M phosphate (pH 7 4) the left with 2 % glutaraldehyde buffered with 0 1 M phosphate (pH 7 4) After decapitation the mucous membranes were removed while in their respective fixatives The right side was then placed in 1° OsO_4 buffered with 0 1 M phosphate (pH 7 4) for 2 hours while the left was prefixed in 2° glutaraldehyde buffered with 0 1 M phosphate (pH 7 4) for 1 hour and post fixed for 1 hour in 1 OsO_4 buffered with 0 1 M phosphate for 1 hour After fixation the tissue was dehydrated in a series of graded alcohols embedded in Epon 812 sectioned with an LKB ultratome stained with uranyl acetate and lead citrate and examined with an RCA EMU 3G microscope

II *Topographical epithelial cell distribution*

Ten healthy squirrel monkeys with good pinneal reflexes were sacrificed by cardiac perfusion with Heidenhain Susa fixative After decapitation the temporal bones were removed decalcified embedded in celloidin and every tenth section was then studied with a light microscope On graph paper (graduated 40 spaces to 10 inches) each horizontal space was considered as representing a section Sections which contained the whole middle ear cavity from the tegmen tympani to the base of the hypotympanum were selected The localizations of cells observed under the light microscope were plotted on the graph paper after actual measurements of distance from Jacobsons n



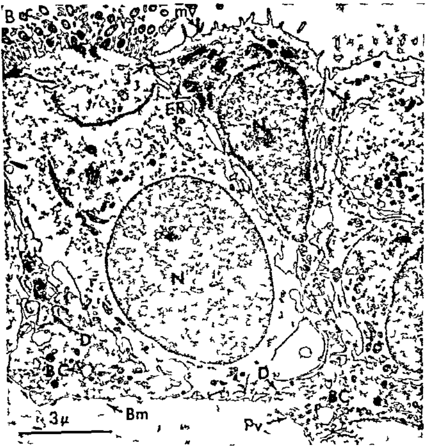
Fig 1 Electron micrograph of tall ciliated cells (*A*) (approx 13 μ) ×4 900 and small ciliated cells (*B*) (approx 11 μ) ×8 100 Along the luminal surface microvilli as well as cilia protrude There are some differences in appearance of microvilli on the ciliated and non ciliated cells The lateral and basal surface adjoining the neighboring cells are conspicuously intricated or interdigitated Small ciliated cells are located among the non ciliated cells Basal cells are located beneath the ciliated and non ciliated cells *N* nucleus *M* mitochondria *Bm* basement membrane *ER* endoplasmic reticulum *mv* microvilli *L* lipid inclusion *Pv* pinocytotic vesicle *c* cilia *BC* basal cell *D* desmosome *J* junctional complex

which served as a reasonably constant reference point A drawing was then made to summarize cell distribution

RESULTS

I *Electronmicroscopic observations*

A basement membrane was seen immediately beneath the epithelial cells Ciliated cells rest on the basement membrane and many cilia with two central and nine peripheral filaments in cross section and many microvilli with complicated shapes which project into the middle ear cavity line their surface The ciliated tall cells (Fig 1 *A*) are usually located in the entrance of the Eustachian tube and hypotympanum as seen in Fig 5 The ciliated small cells (Figs 1 *B* 2) are found among the non ciliated columnar or cuboidal cells Infrequently ciliated small cells are located among the flat cells Within their cytoplasm there are the usual organelles nucleus mitochondria well developed Golgi apparatus moderate amount of RNP particles small round or irregular



shaped vacuoles (smooth and rough surfaced endoplasmic reticulum) Along the basal sur faces of the cells pinocytotic vesicles are some times found Infrequently lipid inclusions with or without pigment bodies were seen (Fig 1 *A*)

Non ciliated cuboidal or columnar cells (Figs *1 B 2*) are found among the small ciliated cells near the entrance of the tube and increase in number towards the posterior middle ear Their cytoplasm has almost the same appearance as ciliated cells but they possess shorter and fewer microvilli than ciliated cells Many fine fila ments were also seen in the cytoplasm

The thin cuboidal and flat cells (Fig 3) have fewer and smaller microvilli than that of ciliated cells and their cytoplasm contains many fine filaments The usual organelles such as mito- chondria Golgi apparatus smooth and rough surfaced endoplasmic reticulum and RNP par ticles are scattered in the cytoplasm however these organelles in flat cells seem to be poorly developed

The lateral surface adjoining the neighbor ing cells is conspicuously intricated or inter digitated especially at the basal half or one third of the lateral wall Approaching the sur face of the middle ear cavity the lateral cell surfaces form the junctional complex which contains the zonula occludence zonula adher ence and desmosome Desmosomes are not usual in this complex but are more commonly seen in the junction between basal cells and other epithelial cells Desmosomes are numer ous and distributed at irregular intervals over lateral cell surfaces Occasionally free wander ing cells were found in the wide intercellular spaces which contain an amorphous electron dense substance (Fig 6)

The basal cells resting usually on the bas ment membrane were found below the ci

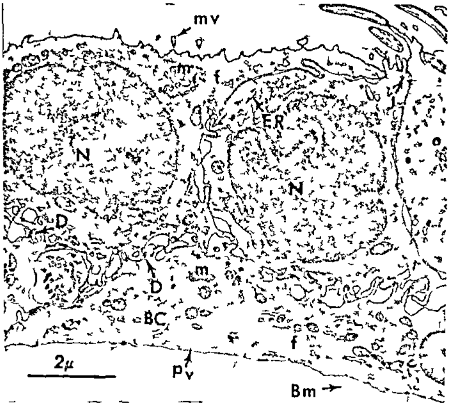


Fig ? Electron micrograph of non ciliated cuboidal cells (approx 7 μ) They possess shorter and fewer microvilli than that of ciliated cells Fine filaments in their cytoplasm as shown in basal cell cytoplasm are found On the basal surface of basal cells many micro pinocytotic vesicles are found *J* junctional complex *D* desmosome *Pv* pinocytotic vesicle *Bm* basement membrane *mv* microvilli *f* filament

d non-ciliated columnar or cuboidal cells These cells interdigitate with desmosomes between adjacent cells Their cytoplasm contains a multitude of fine filaments with the usual cytoplasmic organelles

Very few of the non-ciliated cells possess dense bodies in the apical portion of the cytoplasm These dense bodies were covered by a limiting membrane and were almost oval or round in shape They vary in size from 0 25 to 0 75 μ (Fig 7) Along the basal surface of the cells especially of basal cells many pinocytotic vesicles were found (Figs 1 *B* 2 3 *A*)

II *Topography of middle ear*

The distribution of epithelial cells is shown in Fig 5 This distribution is made by overlying reconstructions obtained from each middle ear Although the distribution of cell groups varied slightly in the many temporal bones observed the following description should be considered as average

The posterior promontory from Jacobson s nerve groove contains flat cells only The anterior promontory from the nerve groove and the region inferior to the round window contains non ciliated cuboidal or columnar cells In certain areas there are clusters or islands of ciliated cells which are small cuboidal or columnar cells as seen by electronmicroscopy The lining of the entrance of the Eustachian tube hypotympanum and the anterior part of the middle ear cavity consistently showed ciliated cells

DISCUSSION

A description of detailed anatomical characteristics of the membrane lining the Eustachian tube middle ear and mastoid is important in determining normal middle ear and Eustachian



Fig 3 Electron micrograph of thin cuboidal cell (*A*) (approx 5 μ) and flat cell (*B*) (approx 3.5 μ) They show small and few microvilli The cytoplasmic organelle of flat cell is poorly developed. *J* junctional complex *f* filament *D* desmosome *g* Golgi apparatus



Fig 4 Electron micrograph of flat cell In this cell there are dense bodies which covered by a limiting membrane *L* lipid inclusion *f* filament *DB* dense body *g* Golgi apparatus

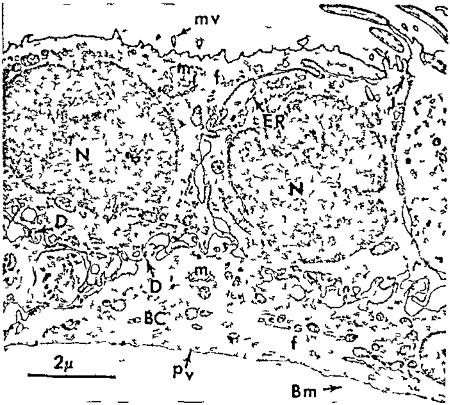


Fig 2 Electron micrograph of non ciliated cuboidal cells (approx 7 μ) They possess shorter and fewer microvilli than that of ciliated cells Fine filaments in their cytoplasm as shown in basal cell cytoplasm are found On the basal surface of basal cells many micro pinocytotic vesicles are found *J* junctional complex *D* desmosome *Pv* pinocytotic vesicle *Bm* basement membrane *mv* microvilli *f* filament

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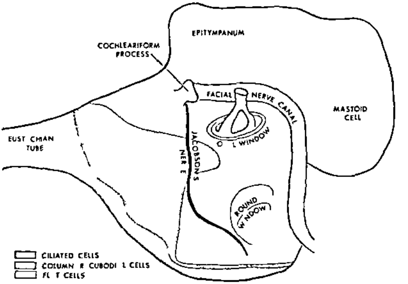


Fig 5 Cell distribution on reconstruction of promontory Ciliated cells are distributed on the entrance tube and hypotympanum Posterior parts from the Jacobson s nerve groove show only flat cells Most of promontory are covered by non ciliated cells

tubal physiology Such a study becomes especially important in interpreting pathological alterations from disease Of special interest in assessing etiology and pathogenesis in man are the evolutionary changes of inflammation ranging from mild (acute serous otitis media) to marked (chronic serous otitis media and mastoiditis)

As a rule ciliated or non-ciliated columnar or cuboidal cells were distributed on the anterior part of the promontory, hypotympanum and in the anterior and posterior middle ear



Fig 6 Electron micrograph cuboidal cells One neutropl leukocyte is found between the epithelial cells *NL* neutrophil leukocyte

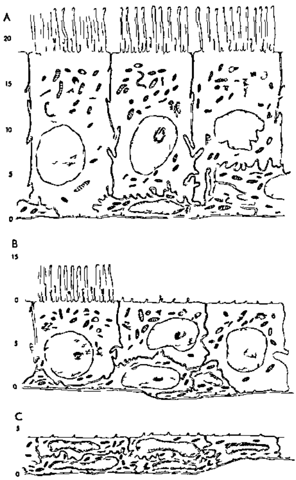


Fig 7 Schematic drawing of cells on the promontory Compare the heights of cells According to the cell heights cells are classified into three types (*A*) 12 μ height (*B*) 5–12 μ height (*C*) 5 μ height

cavity From electron microscopic observations of their cytoplasm these cells are considered to have higher metabolic activity than flat cells Histochemical studies of middle ear mucosa revealed that the high activity of certain oxidative and hydrolytic enzymes were found in ciliated cells while other cell types showed only slight activity (Hiraide & Paparella 1970)

Many observations concerning the presence of ciliated cells in the mucous membrane of the middle ear cavity have been reported (Buch & Jørgensen 1964 Hussel & Lim 1969 Kawabata & Paparella 1969 Lim & Hussel 1969 Sade 1966 Senturia et al 1962) Ciliated cells seem to have a role in clearance of the middle ear cavity and also the capability of absorption since they have cilia and many microvilli with intricate appearances on their surfaces as reported in the trachea (Ross & Rawson 1935)

Goblet cells in the tube and entrance of the tube are frequently found but no gland like structure as reported by Sade (1966) could be located throughout the entire membrane of the middle ear cavity We found lipid inclusions and some lysosome like granules in cytoplasm in some epithelial cells such as Kawabata & Paparella (1969) reported in human cases Although the normal lining of the middle ear was found lacking in mucous secreting elements certain reports (Hilding 1957 Lim & Hussel 1969 Lim et al 1967 Sade 1966) purportedly demonstrated secretory or mucous producing elements besides goblet cells in the middle ear mucosa of guinea pig and human cases They were suggested as performing a secretory function in the middle ear mucosa under normal conditions These suppositions were not confirmed by this study

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We would like to thank Miss Pat Schachern Mrs Kathy Lauhala and Mrs Ann Crowell for their technical assistance

ZUSAMMENFASSUNG

Die spezielle Cytologie des Epithels und dessen Cytotopographie im Mittelohr beim Totenkopfaffchen (Saimiri sciurea) wird hier beschrieben Die folgenden Zelltypen wurden aufgefunden 1) hohe Flimmerzellen 2) kleine Flimmerzellen 3) nicht Flimmer tragende Zylinderepithel oder kubische Zellen 4) dunne nicht Flimmer tragende kubische Zellen oder flache Zellen Flimmerzellen wurden in der Tuba Eustachii und in den vorderen Abschnitten des Mesotympanum wie Hypotympanum angetroffen Zylinderepithel wurde vor dem Jacobsonsche Nerv aber flache Zellen hinter diesem Nerv gefunden Der Letztere diente als Markierung Solche Beobachtungen erscheinen wichtig für das Verstandnis der normalen Physiologie aber ihre Kenntnis ist unerlässlich fur die Deutung krankhafter entzundlicher Veranderungen der Mittelohres aller Grade bei Mensch und Tier

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THE ROLE OF PERILYMPH IN THE RESPONSE OF THE SEMICIRCULAR CANALS TO ANGULAR ACCELERATION

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Abstract A new model for the response of the semicircular canals to angular motion is postulated This model is based on evidence that the bony canal is not compartmentalized and assumes that the ampulla wall is highly flexible It is shown that the perilymph induces a cupula displacement far greater than that produced by the endolymph alone The predicted dynamic behavior of the canals on the basis of this model is found to be consistent with experimental observations

Steinhausen (1927 1931 1933 1939) proposed that the semicircular canals respond to angular accelerations in the same manner as would a heavily damped torsion pendulum According to his model an angular acceleration of the head causes the bony canals and the membranou structures attached to them to accelerate in a similar manner The endolymph within the membranous system however due to its inertia lags behind the motion of the head Because of the small caliber of the membranous canal this relative motion of the fluid is retarded by a relatively large viscous force The cupula which according to his observations fills the entire cross section of the ampulla exerts a restoring force on the fluid when displaced

The mathematical formulation of this idea which in the literature has come to be known as the torsion pendulum equation may be written in the form

$$\Theta\ddot{\xi}+\Pi\dot{\xi}+\Delta\xi=-\Theta\alpha \qquad (1)$$

or

$$\ddot{\xi}+\frac{\Pi}{\Theta}\dot{\xi}+\frac{\Delta}{\Theta}\xi=-\alpha \qquad (2)$$

where ξ $\dot{\xi}$ $\ddot{\xi}$ represent respectively the angular displacement angular velocity and angular acceleration of the endolymph with respect to the skull and where α is the component of the angular acceleration vector perpendicular to the plane of the canal and is measured with respect to an inertial reference frame Θ is an inertia term defined by the mass and distribution of the endolymph The damping coefficient Π denotes the ratio of the torque resulting from viscous forces to the mean angular velocity $\dot{\xi}$ of the endolymph Δ represents the stiffness of the cupula

The angular displacement of the endolymph ξ which is proportional to cupular deflection is also assumed to be proportional to the sensation of angular velocity as well as to the slow phase velocity of nystagmus With this hypothesis it is possible to experimentally determine the constants in the torsion pendulum equation since we can quantify approximately the sensation of angular velocity and can record the nystagmus induced by an angular acceleration stimulus The first to do this were van Egmond et al (1949) Using subjective se o

[1] Dr Van Buskirk is now at the Tulane University School of Medicine This work was carried out at the Ames Research Center of NASA under a collaborative research agreement with Stanford University (NASA grant NGL 05-0 0-223) and was supported in part by the Office of Naval Research under contract number N00014 67 A-0112 0007

as their variable they found $\Pi/\Theta = 10\ \text{sec}^{-1}$ and $\Delta/\Theta = 1\ \text{sec}^{-}$ Niven & Hixson (1961) moni toring nystagmus found values of the same order of magnitude Other experimenters have con firmed these results

Meanwhile as more accurate measurements of the dimensions of the canals became available the ratio Π/Θ could also be determined from the anatomical data Θ the moment of inertia of the endolymph ring can be approximated by the expression

$$\Theta \approx 2\varrho\pi\ r\ R^3 \qquad (3)$$

where ϱ is density of the endolymph r the average radius of the canal duct and R the average radius of curvature of the membranous canal If we assume that the endolymph has a parabolic velocity profile as in the case of Hagen Poiseuille flow in a straight pipe Π may be approximated by the expression

$$\Pi \approx \frac{\varphi}{2\pi} 16\mu\pi\ R^3 \qquad (4)$$

where μ is the viscosity coefficient $\varphi/2\pi$ is that portion of the circumference occupied by the arrow duct The viscous forces in the utricle re insignificant since there the radius is much larger and correspondingly the flow velocities much smaller With $\varphi/2\pi = 2/3$

$$\frac{\Pi}{\Theta} \approx \frac{32\mu\pi\ R^3}{6\varrho\pi^2 r\ R^3} = \frac{32\mu}{6\varrho r} \qquad (5)$$

Using the following anatomical data

$r = 0\ 016$ cm
$\varrho = 1\ 0$ gm/cm³
$\mu = 0\ 852$ centipoise

} (Steer 1967)

one finds $\Pi/\Theta = 180\ \text{sec}^{-1}$ This value for Π/Θ is an order of magnitude larger than that observed experimentally To date no convincing explanation for this discrepancy has been offered

Rejto (1939) proposed that the perilymph might play a more significant role in semi circular canal mechanisms than the endolymph He referred in his paper to the work of two ear lier experimenters namely Spitzer (1925) and Lorente de No (1928) who also held this view Their assumption was in essence that the walls of the ampulla are sufficiently flexible to be deflected by a flow of perilymph Because of the larger size of the perilymphatic space flow ing perilymph would experience a lesser viscous force and hence be much more mobile than the endolymph The basic mechanism is illustrated in Fig 1

The idea of a possible flow of perilymph was vigorously opposed by Dohlman (1941) He argued that his experiments indicated that the membranous ampulla wall was sufficiently rigid to preclude any flow of perilymph How ever his experiments on the ampulla wall were performed after the perilymph was drained In the absence of the normal perilymphatic pres sure the membranous labyrinth could have been excessively pressurized and thus much stiffer than under normal conditions

Results from theoretical and experimental wave transmission studies on canal duct models (Anliker & Dorfman 1970 Anliker & Van Buskirk 1970) clearly indicate that the effects of viscosity of the labyrinthine fluids cannot be disregarded in a realistic study of the dynamic properties of the semicircular canals In view of the small dimensions of the vestibular ap paratus the role played by the viscous forces should be particularly important in the mem branous canal which has an average cross sectional radius of only about 0 16 mm in man Since the cross section of the bony canal is an order of magnitude larger than that of the membranous canal it seems that the peri lymph should indeed be considerably more mobile than the endolymph if the bony canal is not compartmentalized and if the ampulla wall can be considered as a flexible membrane If this were true the motion of the perilymph as well as that of the endolymph would contri bute to the displacement of the cupula To assess the possible significance of this observa tion we are presenting here an elementary theo retical study of a model simulating the gross features of the canals including the ampulla utricle and cupula

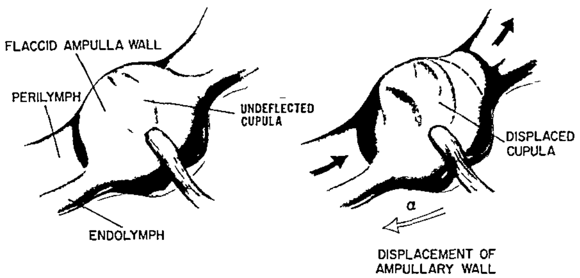


Fig 1 An illustration of the possible mechanics of cupular deflection A flow of perilymph displaces the ampulla wall which in turn produces a cupular deflection Fluid continuity is maintained by an equal and opposite displacement of the ampulla wall on the other side of the cupula

ANATOMICAL FEATURES

According to a biometric study by Igarashi (1966) we can draw a cross sectional view of the horizontal semicircular canal as illustrated in Fig 2 The membranous canal duct runs along the outer periphery of the bony canal where it is secured by fibrous banding to the periosteum (*Gray s Anatomy* 1967) As shown the membranous ampulla takes up the entire cavity formed by the bony ampulla The two ampullae seem also to be connected with fibrous banding (Igarashi 1969) Although the bony canal duct is a confluent channel there is no evidence of perilymphatic cisterns above the membranous ampulla (Igarashi 1969) This implies that any perilymphatic flow which could occur within the bony canal duct cannot extend past the membranous ampulla As the central hypothesis for this analysis we assume now that the sides of the membranous ampulla are flaccid or sufficiently flexible to permit the development of a small net flow of perilymph which theoretically can cause a displacement of the cupula without actually requiring endolymph flow in the membranous canal duct (Fig 1) To maintain perilymph continuity there would have to be an equal and opposite volumetric displacement of the ampulla wall into the perilymphatic space on the opposite side of the cupula

The flow of the perilymph as well as that of the endolymph is resisted by viscous forces which are strongly influenced by the geometric features of the canal ducts A cross section of the duct (after *Gray s Anatomy* 1967) is shown in Fig 3 The location of the membranous canal along the outer periphery of the bony canal is again evident and the relative size of the two canals is clearly illustrated The shape of their cross sections can be approximated by ellipses

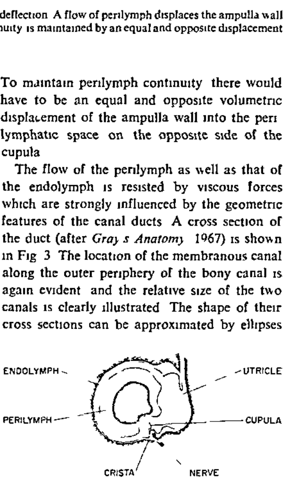


Fig 2 A cross sectional view of the horizontal semicircular canal of man (After Igarashi 1966)

as their variable, they found $\Pi/\Theta = 10\ \text{sec}^{-1}$ and $\Delta/\Theta = 1\ \text{sec}^{-}$ Niven & Hixson (1961) moni toring nystagmus found values of the same order of magnitude Other experimenters have con firmed these results

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and the elastic torques acting on the endolymph and perilymph are respectively

$$T = A_e R\,\delta p \tag{8}$$

and

$$T_p = A_p R_p\,\delta p \tag{9}$$

If we disregard the viscous forces in the regions of the bony vestibule and utricle the equations of motion of the perilymph and endolymph can be written as

$$O\,\ddot\xi + \Pi\,\dot\xi + \Delta_{ee}\,\xi + \Delta_{ep}\,\xi_p = -O\,\alpha \tag{10}$$

and

$$O_p\ddot\xi_p + \Pi_p\dot\xi_p + \Delta_{pe}\,\xi + \Delta_{pp}\,\xi_p = -O_p\alpha \tag{11}$$

where ξ_i denotes the mean angular displacement of the particular fluid ($i=e$ refers to endolymph $i=p$ to perilymph) and where

$$O_i = 2\varrho\pi A_i R_i^2$$

$$\Pi_i = 16\mu_i\pi\, R_i^3\left(\frac{\varphi_i}{2\pi}\right)$$

and

$$\Delta_{ij} = K_c A_i R_i A_j R_j$$

The coefficients in equations (10) and (11) are determined using the following data for the human semicircular canals

A $= 8\,3 \times 10^{-4}$ cm (Igarashi 1966)
A_p $= 110 \times 10^{-4}$ cm (estimate)
R $= 0\,32$ cm (Igarashi 1966)
R_p $= 0\,30$ cm (estimate)
ϱ_i $= 1\,0$ gm/cm³ (Steer 1967)
μ $= 0\,852 \times 10^{-}$ poise (Steer 1967)
μ_p $= 0\,802 \times 10^{-}$ poise (Steer 1967)
$\varphi/2\pi = 2/3$ (estimate)
$\varphi_p/2\pi = 0\,6$ (estimate)

With these values the differential equations of motion are given by

$$\ddot\xi + 170\,\dot\xi + (4\,1 \times 10^{-5})K\,\xi_e + (490 \times 10^{-5})K\,\xi_p = -\alpha \tag{12}$$

and

$$\ddot\xi_p + 11\dot\xi_p + (4\,7 \times 10^{-4})K_c\,\xi_e + (560 \times 10^{-4})K\,\xi_p = -\alpha \tag{13}$$

The stiffness of the cupula K_c is unknown and therefore retained as a parameter Assuming ξ_e and ξ_p to be of the same order of magnitude we can drop the third term in (12) and (13) and reduce them to the form

$$\ddot\xi + \frac{\Pi}{O}\dot\xi + \frac{\Delta_{ep}}{O}\xi_p = -\alpha \tag{14}$$

and

$$\ddot\xi_p + \frac{\Pi_p}{O_p}\dot\xi_p + \frac{\Delta_{pp}}{O_p}\xi_p = -\alpha \tag{15}$$

This system of equations is now solved for a representative function $\alpha(t)$

A typical head movement of short duration can be approximated by an abrupt onset of a constant angular velocity followed by an equally abrupt cessation The angular acceleration and deceleration $\alpha(t)$ associated with such a movement is defined as a positive Dirac delta function $\delta(t)$ at the beginning of the motion and a negative one at its termination Equation (15) stipulates that the perilymph motion has the same character as that of a damped pendulum Since physiological evidence suggests that the motion of the cupula is overdamped it follows that the perilymph motion would also have to be overdamped which implies

$$\Delta_{pp} < \frac{\Pi}{4O_p} \tag{16}$$

If the system is initially at rest and if $\alpha(t) = \delta(t)$ we have as approximate solution of (14) and (15)

$$\xi_p(t) \approx -\frac{\exp\left(-\frac{\Delta_{pp}}{\Pi_p}t\right)}{\frac{\Pi_p}{O_p}} \quad \text{for } t>0 \tag{17}$$

and

$$\xi(t) \approx -\frac{1}{\frac{\Pi}{O}} \quad \text{for } t>0 \tag{18}$$

(A derivation of (17) and (18) is given in the Appendix)

From the solutions (17) and (18) we see that the almost immediate displacement

the endolymph and perilymph are inversely proportional to the respective coefficients Π_e/O_e and Π_p/O_p For the anatomical para meters listed we have therefore $\xi_p > 15\xi$ after the onset of head movement which appears to justify ignoring the third term in equations (12) and (13) If we rotate the head for only a very short duration the exponential decay of ξ_p is negligible Finally when head rotation is terminated the two fluid systems receive a negative impulse which essentially returns them to their original positions

According to equation (6) the volumetric displacement of the cupula is (after inserting anatomical values)

$$\delta V = (2.7 \times 10^{-4})\xi_e + (32 \times 10^{-4})\xi_p \qquad (19)$$

With $\xi_p > 15\xi_e$ for the typical head movement just discussed the contribution of the perilymph to the volumetric displacement of the cupula is almost 200 times that of the endolymph Other acceleration patterns produce similar results which suggests that the cupula displacement is *primarily caused by the perilymph and not by* the endolymph If the contribution of the endo lymphatic motion to the cupula displacement is ignored the governing equation for the response of the perilymph or the cor ponding cupula displacement reduces to

$$\ddot{\xi}_p + 11\,\dot{\xi}_p + 0.056\ K_e\,\xi_p = -\alpha \qquad (20)$$

This means that the response of the cupula is still characterized by a torsion pendulum equation but with the significant difference that the bony canal parameters rather than those of the membranous canal define the dynamics of the system

DISCUSSION AND CONCLUSIONS

The findings of this study may be summarized as follows

1 By assuming that the ampulla wall offers no resistance to small displacements it was shown that it is primarily the perilymph which causes the deflection of the cupula

2 When we allow for perilymph displace ment the equation of motion of the system re tains the form of the torsion pendulum equa tion with the variable ξ now representing the angular displacement of the perilymph rather than endolymph

3 Based on the dimensions of the perilym phatic space the theoretically determined coef ficient Π/O in the torsion pendulum equation is approximately 11 sec^{-1} while the dimensions of the membranous canal lead to a value of about 170 sec^{-1} for the same term

Experimental determination of Π/O through measurements of the slow phase velocity of nystagmus and through subjective sensations of angular velocity leads to values for Π/O which are of the order of 10 sec^{-1} The agreement between the experimentally determined values for Π/O and that predicted by our model lends support to the hypothesis that the ampulla wall offers little or no resistance to displacements and thereby allows the perilymph to produce cupular deflection The validity of this hypo thesis though can only be determined through experimentation on the ampulla wall itself The results of this investigation appear to sug gest a thorough re examination of the nature of the ampulla wall

APPENDIX

Derivation of approximate perilymph and endolymph response to typical head movement

Assuming the system to be initially at rest and $\alpha(t) = \delta(t)$ (Dirac delta function) we can write for the Laplace transform of (15)

$$s^2 x_p(s) + \frac{\Pi_p}{O_p} s x_p(s) + \frac{\Delta_{pp}}{O_p} x_p(s) = -1$$

from which follows

$$x_p(s) = \frac{-1}{s^2 + \dfrac{\Pi_p}{O_p} s + \dfrac{\Delta_{pp}}{O_p}}$$

In view of (16) we may replace the last equation by

$$x_p(s) \approx \frac{-1}{\left(s+\frac{\Delta_{pp}}{\Pi_p}\right)\left(s+\frac{\Pi_p}{O_p}\right)} \qquad (15)$$

The corresponding inverse transform is

$$\xi_p(t) \approx \frac{\exp\left(-\frac{\Pi_p}{O_p}t\right)-\exp\left(-\frac{\Delta_{pp}}{\Pi_p}t\right)}{\frac{\Pi_p}{O_p}-\frac{\Delta_{pp}}{\Pi_p}}$$

By again making use of (16) we can further reduce $\xi_p(t)$ to

$$\xi_p(t) \approx -\frac{\exp\left(-\frac{\Delta_{pp}}{\Pi_p}t\right)}{\frac{\Pi_p}{O_p}} \quad \text{for } t>0 \qquad (17)$$

For $\alpha(t)=\delta(t)$ and the system initially at rest the Laplace transform of equation (14) is

$$s^2 x\,(s)+\frac{\Pi}{O}s x\,(s)+\frac{\Delta_{ep}}{O}x_p(s)=-1$$

Substituting (15) for $x_p(s)$ we find

$$x\,(s) \approx \frac{\frac{\Delta_{ep}}{O_e}}{s\left(s+\frac{\Pi}{O}\right)\left(s+\frac{\Delta_{pp}}{\Pi_p}\right)\left(s+\frac{\Pi_p}{O_p}\right)} - \frac{1}{s\left(s+\frac{\Pi}{O}\right)}$$

and

$$\xi\,(t) \approx \frac{\frac{\Delta_{ep}}{O}}{\left(\frac{\Pi}{O}\right)\left(\frac{\Delta_{pp}}{\Pi_p}\right)\left(\frac{\Pi_p}{O_p}\right)} - \frac{\frac{\Delta_p}{O}\exp\left(-\frac{\Pi}{O}t\right)}{\left(\frac{\Pi}{O}\right)\left(\frac{\Pi}{O}-\frac{\Delta_{pp}}{\Pi_p}\right)\left(\frac{\Pi}{O}-\frac{\Pi_p}{O_p}\right)}$$

$$-\frac{\frac{\Delta_p}{O}\exp\left(-\frac{\Delta_{pp}}{\Pi_p}t\right)}{\left(\frac{\Delta_{pp}}{\Pi_p}\right)\left(\frac{\Delta_{pp}}{\Pi_p}-\frac{\Pi}{\Delta}\right)\left(\frac{\Delta_{pp}}{\Pi_p}-\frac{\Pi_p}{O_p}\right)} - \frac{\frac{\Delta_p}{O}\exp\left(-\frac{\Pi_p}{O_p}t\right)}{\left(\frac{\Pi_p}{O_p}\right)\left(\frac{\Pi_p}{O_p}-\frac{\Pi}{O}\right)\left(\frac{\Pi_p}{O_p}-\frac{\Delta_{pp}}{\Pi_p}\right)} - \frac{1-\exp\left(-\frac{\Pi_e}{O}t\right)}{\frac{\Pi}{O_e}}$$

Except for very small values of t the first four terms on the right hand side of $\xi\,(t)$ are negligible since

$$\frac{\Delta_{pp}}{\Pi_p}<\frac{\Pi_p}{4O_p} \quad \frac{\Pi_p}{O_p}\approx\frac{\Pi}{15O_e} \quad \text{and} \quad \frac{\Delta_{ep}}{O_e}\approx\frac{\Delta_{pp}}{9O_p}$$

Therefore

$$\xi\,(t) \approx -\frac{1-\exp\left(-\frac{\Pi}{O}t\right)}{\frac{\Pi}{O_e}} \quad \text{for } t>0$$

However with

$$\frac{\Pi}{O}\approx 170 \quad \exp\left(-\frac{\Pi}{O}t\right)$$

also approaches zero rapidly and we can thus conclude that ξ_e almost instantaneously assumes the approximate value

$$\xi\,(t)\approx -\frac{1}{\frac{\Pi}{O}} \quad \text{for } t>0 \qquad (18)$$

ZUSAMMENFASSUNG

Es wird ein neues Modell postuliert fur die durch Rotation induzierte mechanische Reaktion des Bogengangappara tes Das Modell basiert auf der Feststellung dass der knocherne Kanal nicht in Kompartimente unterteilt ist und auf der Annahme dass die Ampullenwande sehr flexibel sind Durch mathematische Analyse wird gezeigt dass die Perilymphe eine viel grossere Cupula Auslenkung erzeugt als die Endolymphe Das vorausgesagte dynamische Verhalten des Bogengangapparates ist in guter Übereinstimmung mit experimentellen Beobachtungen

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COMPARISON OF SUBJECTIVE RESPONSES TO SEMICIRCULAR CANAL STIMULATION PRODUCED BY ROTATION ABOUT DIFFERENT AXES

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Abstract A procedure has been developed for obtaining reliable measures of sensation associated with semicircular canal stimulation Theoretically these measures can be used along with measures of nystagmus to estimate several vestibular response system parameters In this experiment responses produced by stimulation of the horizontal semicircular canals are compared with those produced by stimulation of the vertical canals Group mean estimates of subjective angular displacement obtained from forty US Navy flight students were approximately accurate for stimulation of both horizontal and vertical canals Significant individual differences were found within the group From the responses obtained mean estimates of vestibular system parameters were calculated The method appears to be a practical means of measuring the K (θ Δ) parameter which has not been assessed in the past due to lack of a suitable method

In recent experiments it was found that men were able to make consistent estimates of angular displacement when they were rotated passively through short arcs about an Earth vertical axis (Guedry et al 1970) Purposely excluded from the test situation were visual and auditory cues to relative motion between the man and the Earth Since these sources of sensory information were absent it is reasonable to assume that the semicircular canals were the primary source of information for these judgments

Stimulus–response relationships such as these can be predicted from the torsion pendulum model proposed by van Egmond et al (1949) and Groen (1956) and insofar as the model is appropriate these relationships provide a means for measuring parameters of individual sensations which may be relevant to the clinical assessment of pilot vertigo (Benson 1967 Guedry et al 1970)

The present experiment was undertaken to extend application of triangular waveform procedures to evaluation of responses produced by stimulation of the vertical semicircular canals and to compare these responses with those produced by stimulation of the horizontal canals in the same set of subjects

METHOD

Subjects

Forty US Navy and Marine officers awaiting flight training served as subjects All had passed standard Navy flight physical examinations without signs of vestibular disorders

Apparatus

The rotation device was a Stille Werner RS-3 rotator fitted with a rectangular platform upon

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Opinions or conclusions contained in this report are those of the authors They are not to be construed as necessarily reflecting the view or the endorsement of the Departments of the Army or Navy

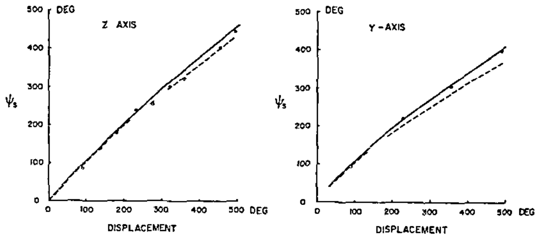


Fig 1 Mean angular displacement estimates when subject is rotated about his z axis (*left*) and his y axis (*right*) Solid lines and solid circles indicate predicted and obtained results respectively for $\alpha = 15$ deg/sec² Dashed lines and open circles indicate predicted and obtained results respectively for $\alpha = 10$ deg/sec²

which a subject could be positioned on his side or seated upright near the center of rota tion

Procedure

Two groups of 20 subjects were selected Sub jects in the first group were tested in complete darkness to prevent their use of visual cues but no effort was made to eliminate auditory or cutaneous cues The second group was also tested in complete darkness and in addition auditory cues were minimized by applying wide band noise through audiometric ear-phones and cutaneous cues were minimized by *encapsulating the subject to reduce air cur-rents*

With the exceptions just noted the proce-dure was the same for both groups Two series of stimuli were presented to each subject Sub jects were seated upright in one series with the z axis[1] and the axis of rotation in alignment to stimulate the horizontal canals in another series subjects were positioned right side-down with the y axis and the rotation axis in align ment to stimulate the vertical canals Half of the subjects were tested in the right side down position first and the other half upright first

[1] The nomenclature system proposed by Hixson et al (1966) is used here

Each series consisted of twenty triangular waveform stimuli Each stimulus waveform comprised angular acceleration and decelera tions of equal magnitude and duration Two accelerations were used 10 and 15 deg/sec² and each was combined with five stimulus durations to give ten displacements that ranged from 90 to 495 deg Each of the ten displace ments was given in both the clockwise and counterclockwise direction Order of presenta tion of stimuli within series was scrambled but was the same for each subject A 30 sec rest period was allowed between stimuli

Judgments of angular displacement (ψ_s) were reported verbally in degrees Subjects were in structed to make retrospective judgments i e to delay judgment until apparent displacement was complete Instructions were specific in this regard to avoid concurrent velocity estimates which according to earlier studies (Guedry et al 1969 Guedry et al 1970 Owens & Gue dry 1969) yield systematic underestimation of larger displacements

RESULTS

Mean subjective displacement judgments (ψ_s) were approximately correct throughout the range of stimuli under all conditions of the ex periment as shown in Fig 1 Correlations be tween actual and perceived displacement for

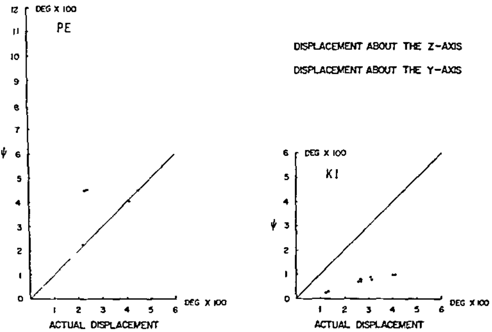


Fig 2 Mean angular displacement estimates from two subjects PE and KI

individual subjects ranged between 0 46 and 0 99 with a mean of 0 89 This differs significantly from a mean of 0 95 found earlier with slightly different methods of reporting (Owens & Guedry 1969)

For purposes of making statistical comparisons the slope of the best fitting straight line through paired values of estimated and actual angular displacement was calculated using a least squares criterion for each subject under each condition of the experiment These slopes were then used as data for an analysis of variance test of statistical significance between experimental conditions The analysis showed that subjects underestimated the larger angular displacements to a greater extent during y axis rotation than during z axis rotation. This difference was statistically significant at $p < 0\ 01$ Also subjects made slightly smaller estimates of the larger displacements with the 10 deg/sec stimuli than with the 15 deg/sec stimuli The difference was not statistically significant in the present sample but it was also observed in a previous experiment (Guedry et al 1970) and was expected on the basis of theoretical considerations discussed below Neither the direction of rotation for a given axis nor the presence of extraneous cutaneous and auditory cues significantly affected displacement judgments

Large differences were found between individuals as illustrated in Fig. 2 by the results of two subjects selected because they represented opposite extremes of the distribution Although they differed from each other each of these individuals gave fairly consistent responses In this respect they were typical of most subjects in the sample Moreover individuals tended to give similar judgments for rotation about both the y and z axes i e a subject who gave large estimates for z axis stimuli also tended to give large estimates for y axis stimuli The slopes of responses for the two axes were significantly correlated (r_y = 0 62 $n = 40$) a result which may indicate that individuals tend to have "matched sets of canals but which may also be indicative of a common nervous system gain factor

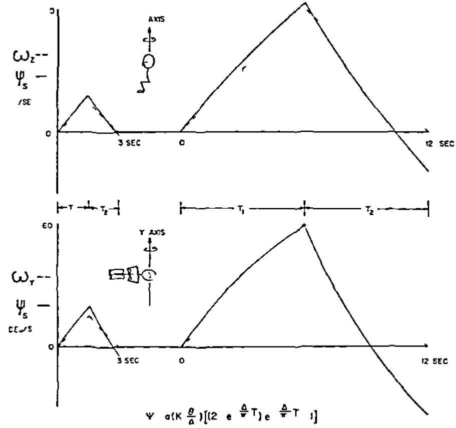


Fig 3 Subjective angular velocity (ψ_s) during triangular waveforms of angular velocity (ω) in the z and y axis series For the z axis response curves $\Pi/\Delta = 16.0$ sec and $K(\theta/\Delta) = 19.9$ sec for the y axis response curves $\Pi/\Delta = 7.2$ sec and $K(\theta/\Delta) = 10.5$ sec

DISCUSSION

As stimulus wavelength was increased displacement estimates in the y axis series (vertical canals) became progressively smaller than displacement estimates in the z axis series (horizontal canals) This difference was statistically significant indicating that the method was sufficiently sensitive to detect differences between the vertical and horizontal canal response systems

The use of angular displacement judgments along with other response measures to determine parameters of these two systems is based on the torsion pendulum model (van Egmond et al 1949 Groen 1956) If it is assumed that subjective angular velocity (ψ_s) is proportional to cupula deflection then ψ_s should increase and decrease during triangular waveforms as shown in Fig 3 The dashed lines represent stimulus angular velocity and the solid lines represent ψ_s According to the equation (derived from the torsion pendulum model) presented in Fig 3 two constants Π/Δ and $K(\theta/\Delta)$ determine the match between stimulus and subjective angular velocity With waveforms like those in the present experiment both constants influence the maximum ψ_s response attained but Π/Δ alone determines the point in time when the response crossed the zero baseline

Theoretically it is this velocity information ψ_s which the subject must integrate over time to make a displacement estimate Mathematical integration of ψ_s from the beginning of the stimulus until the response crosses its zero baseline gives the area under the response curve which should predict displacement estimates (ψ)

$$\psi = \alpha\left(K\frac{\theta}{\Delta}\right)\left[T_1 - \frac{\Pi}{\Delta}\ln\left(2-\exp\left(-\frac{\Delta}{\Pi}T_1\right)\right)\right]$$

where θ Π and Δ are the inertial viscous damping and spring constants of the cupula endolymph system K_s is a constant of proportionality between cupula deflection and ψ_s α

and T_1 are respectively magnitude and duration of angular acceleration

This equation was used to generate the smooth curves in Fig 1 and provides a close fit to obtained data Values of $K\ (\theta/\Delta)$ −19 9 sec and $\Pi/\Delta=16$ sec were used in Eq (1) to fit the z axis data These same values provided a close fit to data in a previous study (Guedry et al 1970) Values of $K\ (\theta/\Delta)=10\ 5$ sec and $\Pi/\Delta=7\ 2$ sec were used to fit the y axis data The Π/Δ values selected for the z and y axes are consistent with values determined by other methods (Benson & Guedry 1970 Jones et al 1964) and although previous estimates of $K_s(\theta/\Delta)$ are not available for the y axis the ratio of the $K\ (\theta/\Delta)$ values for the y and z axes is consistent with an analogous ratio derived from nystagmus data presented by Collins & Guedry (1967) Thus it appears from several sources that there is a clear difference in the Π/Δ parameter for z and y axis responses but that there is also a compensatory difference in the $K_s(\theta/\Delta)$ constants which works to approximately equate subjective velocity information for z and y axes when the stimulus waveforms are short With longer waveforms there are theoretical differences in responses about those two axes which were reflected in the obtained data Responses to z and y axis stimuli differed in another way subjects spontaneously reported that the aftereffects of y axis stimuli were greater than the aftereffects of z axis stimuli This difference would be expected from the relative overshoots of the z and y axis responses shown in Fig 3

The results of the present study as well as previous results (Guedry et al 1969 Owens & Guedry 1969) illustrate that man can integrate the available sensory information provided by triangular waveform stimuli to give approximately accurate and consistent estimates of subjective angular displacement (ψ) when he makes retrospective judgments However the subjects in this experiment made less consistent judgments than did those in a previous experiment (Guedry et al 1970 Owens & Guedry 1969) and perhaps this was due to a difference in procedure In the earlier study (Owens & Guedry 1969) the interior of the capsule was dimly illuminated and estimates were made by manual displacement of a pointer on a circular dial marked off in angular units whereas in the present study the chamber was dark and subjects gave verbal estimates of displacement Testing in darkness permits the simultaneous recording of nystagmus and this practical advantage probably offsets the slight loss of consistency of estimates However when the individuals tested are less familiar with units of angular measure than were the aviation personnel in the present studies then the former method may be necessary

As indicated earlier (Guedry et al 1970) triangular waveforms of angular velocity can be used to determine both the Π/Δ and $K\ (\theta/\Delta)$ constants for individuals from nystagmus as well as subjective responses and they may also be a means of determining adaptation time constants (Guedry et al 1970) The methods proposed seem to offer some practical advantages in regard to reliability and ease of measurement If the theory upon which they are based is adequate then the parameter measured should be valuable clinical tools

ACKNOWLEDGMENTS

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ZUSAMMENFASSUNG

Ein Verfahren zur Gewinnung zuverlassiger Messwerte fur die mit der Reizung der Bogengange verbundenen Empfindung ist entwickelt worden Diese Messwerte konnen zusammen mit den Messresultaten fur den Nystagmus benutzt werden um mehrere Parameter der vestibularen Reaktion annahernd zu bestimmen In diesem Versuch wurden Reaktionen die durch Reizung des horizontalen Bogenganges hervorgerufen wurden mit solchen des vertikalen Bogenganges verglichen Gruppenmittelwerte der geschatzten subjektiven Winkeldrehung von vierzig Marineflugschulern waren angenahert richtig bei Reizung des horizontalen und senkrechten Bogenganges In der Gruppe wurden signifikante individuelle Unter

schiede beobachtet. Aus den erhaltenen Reaktionen wurden geschatzte Mittelwerte der vestibularen Systemparameter berechnet. Die Methode erweist sich als ein zuverlassiges, praktisches Verfahren zur Messung des Λ (θ/Λ) Parameters der bisher infolge Fehlens einer brauchbaren Methode nicht ermittelt werden konnte

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THE LOADING EQUILIBRIUM EXAMINATION FOR ETIOLOGIC DIAGNOSIS OF MENIÈRE S DISEASE

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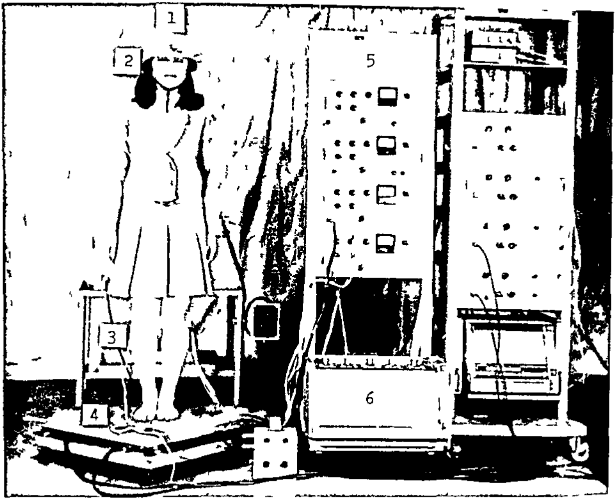
Abstract Loading equilibrium examinations were carried out to diagnose the etiologic factors in the labyrinthine disturbance in individual cases of Menieres disease The examinations were designed to estimate the variations of labyrinthine function provoked by loading to the circulatory autonomic nervous or metabolic system of the patients through equilibrium examinations such as ataxiagram writing test stepping test, and/or electronystagmogram When the loading induced disturbance of labyrinthin function dysfunction of the system loaded was judged to be a significant etiologic factor for Menieres disease

As a result equilibrium was disturbed by loading of standing in 9 of 12 cases by pressure upon the eyeballs in 33 of 46 cases by pressure upon the carotid sinus in 21 of 37 cases by injection of the autonomic drugs i e by adrenalin in 29 of 42 cases by pilocarpine in 28 of 42 cases and by atropine in 21 of 42 cases by histamine injection in 4 of 15 cases by water loading in 31 of 58 cases and by neck torsion in 11 of 30 cases Attacks of vertigo were induced in 2 cases each by water loading and pilocarpine injection The loading equilibrium examination was useful both for the etiologic diagnosis of Menieres disease and for the choice of therapy

There have been a number of theories advanced based on a great deal of experimental and clinical evidence concerning the cause of Menieres disease The causative factors described for the condition include autonomic dysfunction (Montandon 1945) allergy (Atkinson 1941) disturbance of water metabolism (Mygind & Dederding 1938) vitamin deficiency (Atkinson 1949 1950 1953) nutritional disturbance (Selfridge 1949) hyperadrenocorticism (Goldman 1962) cervical disorders (Wildhagen 1958) and emotional stress (Fowler & Zeckel 1952) This suggests that Menieres disease may be an ailment of the inner ear caused by a variety of systemic abnormalities

It is always necessary however to elucidate the cause of the disturbance of the inner ear in individual patients with Menieres disease in order to prescribe proper medical treatment The difficulty is to determine which of these many causative factors is of etiologic significance in the disturbance of the inner ear in individual cases Even if abnormal blood pressure or autonomic dysfunction has been revealed by examination these abnormalities might not be directly related to the pathogenesis of the condition since abnormal blood pressure or autonomic dysfunction can be present either as a symptom of Menieres disease or independently of the condition Thus the etiologic diagnosis of Menieres disease should be based on clear demonstration of the relationship of general disturbance to labyrinthine disorder

The authors carried out loading equilibrium examinations to elucidate the relationship of the general disturbance to Menieres disease The examination consisted of a loading to the autonomic nervous system circulatory system or metabolic system to estimate by equilibrium examination the resultant variation in labyrinthine function and thus to clarify the relationship of various general disturbances to labyrinthine disorder The examination was an attempt to provide a guide to the medical treatment of the disease based on the causative factor determined for each case



g 1 Ataxiagraphy simultaneously recording of Gs swaying of the head and the center of gravity nd EMGs of the anterior tibial and gastrocnemius muscles 1 Helmet to which 2 accelerometers are attached 2 Electrodes for recording electronystagmogram 3 Electrodes for recording electromyograms of ant tib and gastrocnemius muscles 4 Stand for recording swaying of the center of gravity Four load cells are placed under the stand 5 Polygraph 6 Recorder

METHOD

Our series consisted of 136 patients diagnosed Meniere s disease at the Dept of Otolaryngology Gifu Medical School during the 7 year period from 1963 through 1969

Loading equilibrium examinations were performed as follows

A Equilibrium examination during and after orthostatic loading to the circulatory system

B Equilibrium examination during and after pressure upon the eyeballs or carotid sinus

C Equilibrium examination after loading with autonomic drugs

D Equilibrium examination after histamine injection

E Equilibrium examination after water loading

F Equilibrium examination during and after neck torsion

Furthermore electroencephalography was carried out in an attempt to search for general abnormalities in cases of Meniere s disease

The following methods were used for equilibrium examination for labyrinthine function

1 Ataxiagram or cephalogram
2 Vertical writing test (Fukuda 1959)
3 Stepping test (Fukuda 1959)
4 Observation of spontaneous nystagmus

An ataxiagram represents a simultaneous recording on a polygraph of the ECG electronystagmogram swaying of the body (swaying of the head and/or the center of gravity) and electromyograms of the anterior tibial and gastrocnemius muscles in a subject standing erect Fig 1 shows the procedures employed Observation of nystagmus and swaying of the body permitted an evaluation of how the body equilibrium was being maintained The number of cases studied varied according to the different examination since each examination was not performed in all cases of our series

OBSERVATIONS

A *Equilibrium examination during and after orthostatic loading to the circulatory system*

Subjects were asked to stand for 10 min to estimate blood pressure during and after orthostatic loading (Schellong s test) while making an observation of equilibrium function In writing test before and after orthostatic loading for 10 min in 10 normal adults deviation of column of letters written in the vertical direction with eyes covered (writing deviation) was observed within the limit of 9 degrees Cephalographically there was no case where the amplitude of swaying of the head after orthostatic loading was one and a half times as large as that prior to the loading

Fig 2 shows the results of the writing test carried out simultaneously with Schellong s test in a 64-year-old male patient with Meniere s disease The column of letters written with the patient blindfold deviated approximately 9 degrees to the right when the patient was sitting with a blood pressure of 142/66 mmHg and deviated 25 degrees to the right as shown in the lower part of the figure when his blood pressure fell to 102/56 mmHg after 10 min of quiet standing Thus his equilibrium function was disturbed by a decrease in blood pressure

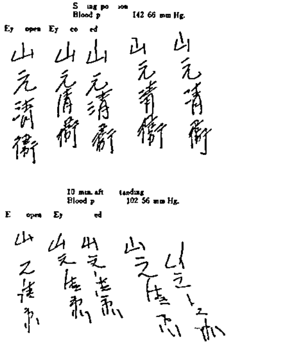


Fig 2 Writing before and after loading of upright standing Meniere s disease 64 year old male showing orthostatic dysregulation

Examination of blood pressure revealed normal pressure in 36 hypotension in 23 orthostatic dysregulation in 23 and hypertension in 2 out of 84 cases of Meniere s disease In 12 of 23 cases of orthostatic dysregulation equilibrium examination combined with Schellong s test was performed The examination revealed a developing or intensified disturbance of equilibrium function in 9 of 12 cases

This examination is useful also in evaluating therapeutic efficiency Fig 3 shows the relationship between the variation in blood pressure and a swaying of the head while in a standing position in a 27 year old female patient with Meniere s disease Swaying of the head marked 7 0 × 3 4 cm immediately after standing up when blood pressure was 120/74 mmHg and was increased to 11 6 × 10 2 cm with the decrease in blood pressure 10 min after standing up Here injection of 3 ml of carnigen (2 mg of adenosine and 10 mg of racemic p-hydroxyphenylmethylaminopropanol hydrochloride per

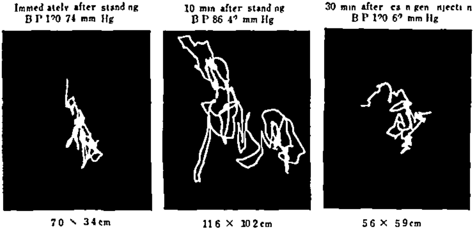


Fig 3 Cephalogram and blood pressure before and after carnigen injection Menieres disease 27 year old female showing orthostatic dysregulation

ml) to obtain a blood pressure of 120/62 mmHg brought improvement in ataxia with the head swaying reduced to 5 6 × 5 9 cm

As discussed above orthostatic dysregulation of blood pressure can be regarded as a significant etiologic factor for labyrinthine disturbance in the cases of Menieres disease where the blood pressure fall may be attended by a disturbance of equilibrium function

Equilibrium examination during and after pressure upon the eyeballs or carotid sinus

Subjects underwent pressure upon the eyeballs or carotid sinus for 20 sec to count the pulse rate before during and after the pressure while also observing the equilibrium function In the writing test during and after pressing on the eyeballs or carotid sinus in 10 normal adults writing deviation was within 9 degrees In ataxiagram swaying of the body was increased after as compared with before pressing in most cases although the amplitude of the swaying was less than one and a half times that before pressing Pressing on the eyeballs or carotid sinus was followed by nystagmus in none of the cases

Fig 4 shows ataxiagram taken with pressure upon the eyeballs in a 29 year old male

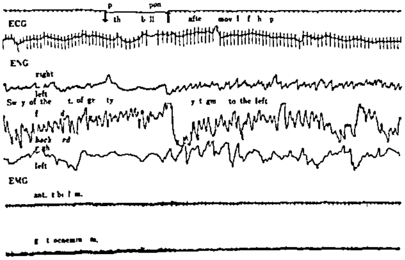


Fig 4 Ataxiagram before during and after pressure upon the eyeballs Menière s disease 29 year old mal

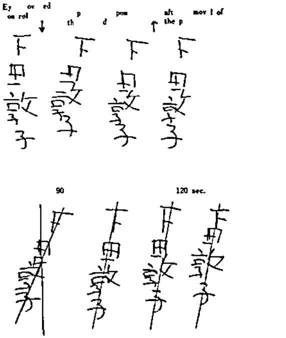


Fig 5 Writing before during and after pressure upon the carotid sinus Meniere s disease 19 year old female

patient with Meniere s disease There was a spontaneous nystagmus to the left even before the pressure Nystagmus was increased both in amplitude and in frequency following the pressure Swaying of the body was also increased as compared with that before the pressure was applied

Equilibrium examination with exertion of pressure upon the eyeballs revealed a developing or increased disturbance of equilibrium function in 33 of 46 cases of Meniere s disease

Fig 5 shows the results of a writing test with pressure upon the carotid sinus in a 19 year old female patient with Meniere s disease The pressure caused pathological deviation to the left of the column of letters written with the eyes blindfolded Equilibrium disturbances due to pressure upon the carotid sinus tended to be more intense when the pressure was exerted on the carotid sinus of that side where the inner ear was already impaired

Equilibrium examination with exertion of pressure upon the carotid sinus revealed a developing or increased disturbance of equilibrium function in 21 of 37 cases of Meniere s disease Disturbance of equilibrium also appeared in some cases with no variation in pulse rate caused by the pressure in the examination

Thus disturbed reflexive regulation of the inner ear blood vessels may be an etiologic factor for labyrinthine disturbance in those cases of Meniere s disease where pressure upon the eyeballs or carotid sinus may lead to an equilibrium disturbance

C *Equilibrium examination after loading with autonomic drugs*

Subjects were injected with 0 1% adrenalin (0 013 ml/kg) 1° pilocarpine (0 013 ml/kg) or atropine (0 013 ml/kg) and equilibrium examinations were performed before and after injections In 5 normal subjects without any abnormal response of the autonomic nervous system to autonomic drug injection writing and stepping tests and cephalography carried out at intervals of 10 30 and 60 min after the injection revealed no writing deviation over 9 degrees no stepping deviation (angle of rotation of the body around its vertical axis in 100 steps) over 45 degrees nor any increase in the amplitude of swaying of the head to one and a half times that before injection Ataxiagraphic observation demonstrated no appearance of spontaneous nystagmus nor any increase up to one and a half times the initial level in swaying of the body

Fig 6 shows the results of ataxiagraphic observation of bodily equilibrium before and after pilocarpine injection in a 50-year-old male patient with Meniere s disease There was a spontaneous nystagmus to the right even before injection Nine minutes after pilocarpine injection there was an increase in the intensity of the nystagmus and in body swaying Fifty minutes after pilocarpine injection spontaneous nystagmus disappeared with decreased swaying of the body in an upright position Namely ataxia was increased transiently and then improved with passage of time after pilocarpine injection Mygind (1952) referred to

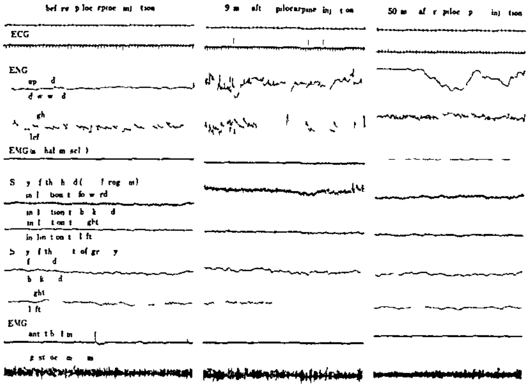


Fig 6 Ataxiagram before during and after pilocarpine injection Meniere s disease 50-year old male

the pilocarpine experiment by Dederding stating that there was a loss of fluid and an increase of hearing but there was also an initial stage of contrary effect with a temporary decrease in hearing and a spontaneous nystagmus He further stated that an initial stage of contrary effects was found with all diuretics and it was due to a temporarily increased intralabyrinthine output and pressure This explanation by Mygind permits our finding that pilocarpine injection caused first a transient aggravation and then an improvement of equilibrium function to become understandable

Equilibrium function decreased after injections of adrenalin pilocarpine and atropine in 29 28 and 21 cases respectively out of 42 cases of Meniere s disease Pilocarpine injection caused paroxysmal vertigo in 2 cases There were some cases on the other hand where equilibrium function was improved by injection of autonomic drugs

Thus autonomic dysfunction may be considered to be the etiologic factor for labyrinthine disturbance in those cases of Meniere disease where equilibrium function is distur by injection of an autonomic drug

D *Equilibrium examination after histamine injection*

Equilibrium examination was carried out l lowing histamine injection in 15 cases Meniere s disease with a history of rhin vasomotorica asthma bronchiale or food lergy The procedure employed was what H ton (1956) used for the diagnosis of histami cephalgia Houton stated that headache dev oping immediately after histamine inject represents a physiologic effect due to the ph macologic activity of histamine and that t symptom may once disappear and then prese itself again as a pathologic response We h accepted this viewpoint in studying equilibri function following histamine injection All n mal adults complained of headache persisti

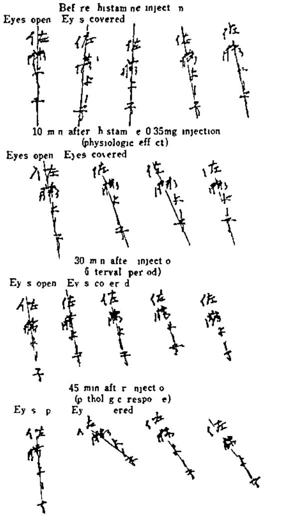


Fig 7 Writing before and after histamine injection Meniere s disease 40 year old female showing food allergy

for 5 to 10 min after histamine injection No equilibrium disturbance was detected by writing and stepping tests or cephalography carried out at intervals of 10 20 30 40 and 60 min after the injection

Fig 7 shows the results of writing tests before and after histamine injection in a 40 year-old female patient with Meniere s disease The column of letters written with eyes blindfolded deviated 24 degrees to the right with a concomitant development of headache 10 min after injection of 0 35 mg histamine (physiologic effects) Deviation of writing reduced to 19 degrees 30 min later but was again as severe as 45 degrees to the right 45 min later (pathologic response) Atkinson (1941) described a case of attack of vertigo induced by a histamine skin test We studied by means of equilibrium examination patients with Meniere s disease who were very sensitive to histamine

The examination revealed pathologic equilibrium disturbances in 4 of 15 cases of the disease These cases were diagnosed as histamine hypersensitivity of the inner ear

E *Equilibrium examination after water loading*

Equilibrium examinations were carried out before and after water loading Our examinations combined observation of equilibrium function using the water loading test (Jadresic et al 1962) which consisted of administration of 1 litre of water and the study of diuresis which ensued during the following 2 hours In 6 normal adults writing deviation was within 9 degrees and stepping deviation was within 45 degrees at 1 and 2 hours after water loading Cephalographically swaying of the head was increased up to one and a half times that before water loading

Fig 8 shows the results of an equilibrium examination with water loading in a 58 year old male patient with Meniere s disease One hour after water loading there was increased swaying of cephalographic pattern with an increase to 110 degrees of angle of stepping deviation Two hours after loading the patient complained of rotatory vertigo with a development of nystagmus falling in the stepping test and increased fluctuation of the cephalographic pattern The attack of vertigo persisted until the next day The amount of urine excreted for 2 hours after water loading in this case was 640 ml the excretion rate being lower than that for healthy subjects (more than 70) Mygind (1952) referred to a case of Meniere s disease where hearing loss tinnitus vertigo vomiting pressure in the ear and headache were produced after a water test We studied by

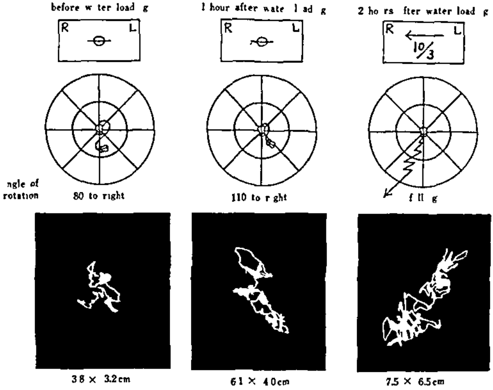


Fig 8 Cephalogram stepping and nystagmus before and after water loading Menieres disease 58 year old female showing decrease of excretion rate of urine Two hours after water loading vertiginous attack was induced

equilibrium examination the disturbances of vestibular function due to water loading

Equilibrium function worsened following water loading in 31 of 58 cases of Menieres disease Attacks of vertigo were noted in 2 cases As to the relationship between the amount of urine excreted and the results of equilibrium examinations there was disturbed equilibrium function in 5 of 7 cases with excretion rates below 55% (abnormal response)

Thus disturbance of water metabolism can be regarded as an etiologic factor in disorder of the inner ear in cases of Menieres disease where equilibrium function may be disturbed by water loading

F *Equilibrium examination during and after neck torsion*

Equilibrium examination with neck torsion was performed in 30 cases of Menieres disease complaining of vertigo due to neck torsion limited rotation of the neck occipitalgia and/or stiff shoulder Writing test under head torsion in 5 normal adults showed that writing deviation was within 12 degrees when the head was fixed in right or left rotated position and within 9 degrees when the head returned to normal position Ataxiagraphically head torsion was not associated with manifestation of nystagmus or an increase in bodily swaying up to a level one and a half times as large as that in normal position

Fig 9 shows an ataxiagram taken with neck torsion in a 50-year old male patient with Menieres disease Neck torsion to the left was followed by occurrence of nystagmus to the left and increase in body swaying The patient had cervical spondylosis of the cervical vertebrae revealed by X ray examination These results depict Menieres disease due to cervical disorder as defined by Wildhagen (1958) Since an abnormality in the cervical vertebrae

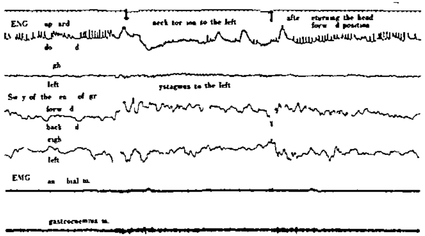


Fig 9 Ataxiagram of before during and after neck torsion Meniere s disease 50-year-old male showing cervical spondylosis in the region of C_4 and C

may exist independent of Meniere s disease it is desirable to clarify the relationship between the two in an objective manner by equilibrium examination with neck torsion

Equilibrium examination with neck torsion revealed developing or increased equilibrium disturbance in 11 of 30 cases of Meniere s disease In these cases cervical disorders were determined to be an etiologic factor for labyrinthine disturbance Morphological abnormalities of the cervical vertebrae were seen radiologically in 9 and an abnormal running of the vertebral artery revealed by vertebral angiography in 2 of 11 cases

G *Electroencephalography*

EEG findings in 70 cases with Meniere s disease are summarized in Table I Recordings were made at intermittent periods of the disease in all cases Brain wave was registered at rest in hyperventilation and under activation with megibal (β β methylethylglutarimide) As to brain wave activation with megibal it has been reported that no abnormal findings of brain wave were produced by intravenous injection of the drug 100 mg in 5 min in non epileptic subjects (Togashi & Yagi 1959)

EEG findings of Meniere s disease are characterized by no abnormality at rest and by appearance of a high voltage slow wave 3–6 per sec rhythmic high voltage wave or irregular spike and wave complex at activation with megibal These findings correspond well to the results of electroencephalographic observation of Meniere s syndrome by Naoi et al (1958) These abnormalities are considered to represent not epilepsy or vertiginous epilepsy (Behrman 1955) but central predisposition to Ménière s disease as pointed out by Naoi and his coworkers Akimoto (1960) stated that abnormal brain wave patterns are of low incidence at rest but frequently produced by provocation method in cases of hemicrania The similarity of EEG findings in Meniere s disease to those of hemicrania suggests that these two conditions may be predisposed by the same factors

Table I *EEG findings of 70 patients with Meniere s disease*

At rest	
Normal EEG	54
Flat EEG	9
Dysrhythmia	1
Diffuse fast wave	4
High voltage alpha wave	2
Activated EEG by hyperventilation	
Build up	1
Activated EEG by megibal	
Normal EEG	38
Dysrhythmia	8
High voltage slow wave	2
Rhythmic high voltage wave	7
Irregular spike and wave	15

CONCLUSION

In the above sections the results of loading equilibrium examination in cases of Meniere s disease were discussed The examination was useful in clarifying the relationship of general abnormality to labyrinthine disturbance in individual cases of Meniere s disease However attacks of vertigo were induced only in 2 cases each by water loading and pilocarpine injection In other cases vestibular function might be disturbed but not leading to attacks of vertigo Consequently even if loading may disturb the function of the inner ear dysfunction of the system loaded can not directly be identified as the only cause of the disturbance of the inner ear Predisposition of the inner ear is considered to be another important factor for the pathogenesis of Meniere s disease or manifestation of vertigo That is abnormalities detected by loading equilibrium examination are likely to be an etiologic factor for or general predisposition to Meniere s disease

The examination was useful in selecting the proper therapy for individual cases of Meniere s disease Sedatives and tranquilizers are effective against vertigo associated with the disease and vasodilatators and vitamins against disturbance of the inner ear The choice of a remedy for the etiologic factor can be based on the results of loading equilibrium examinations

Fukuda & Tokita (1963) stated equilibrium examination for detection of disturbance of the inner ear should include (1) tests related to righting reflexes (2) tests related to spontaneous deviation and (3) tests related to induced labyrinthine nystagmus Tests (1) and (2) are necessary to determine vertigo or ataxia in an objective manner and test (3) to examine labyrinthine responsiveness We consider that loading equilibrium examination should be added as part of this series of examinations

ZUSAMMENFASSUNG

Ladegleichgewichtsversuche wurden ausgeführt um die etiologischen Faktoren bei Storungen im inneren Ohr bei einzelnen Fällen der Menièreschen Krankheit zu diagnostizieren Bei den Versuchen wurden das Blutkreislaufsystem das autonome Nervensystem oder das Stoffwechselsystem der Patienten geladen um die daraus entstehenden Variationen der Labyrinthfunktion durch Gleichgewichtsversuche wie zum Beispiel Ataxiagramm Schreibe-Versuch Tretversuch und/oder Elektronystagmogramm zu bestimmen Wenn das Laden Storungen der Labyrinthfunktion hervorrief wurde dies als ein verlasslicher etiologischer Faktor der Meniereschen Krankheit angesehen Das Gleichgewicht war gestort bei Standladung in 9 von 12 Fällen bei Druck auf die Augapfel in 33 von 46 Fallen bei Druck auf den Karotis Sinus in 21 von 37 Fallen bei Injektion einer autonomen Droge dh Adrenalin in 29 von 42 Fallen durch Pilokarpin in 28 von 42 Fallen und durch Atropin in 21 von 42 Fallen durch Injektion von Histamin in 4 von 15 Fällen durch Wasserladung in 31 von 58 Fällen und durch Kopfdrehung in 11 von 30 Fallen Schwindelanfälle wurden in vier Fallen zweimal durch Wasserladung und zweimal durch Pilokarpininjektion hervorgerufen Die Ladgleichgewichtsuntersuchung war sowohl für die etiologische Diagnose der Menereschen Krankheit als auch fur die Wahl der Therapie von Nutzen

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PSYCHOSOMATIC ASPECTS OF MENIERE S DISEASE

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Abstract It has been known by medical authorities for many years that patients who suffer from Meniere s disease are subject to attacks of anxiety and elevated emotional tensions This paper attempts to illustrate the possibility that the onset of Meniere s symptoms can be linked to life stress situations In the past thirty five years several carefully controlled studies have been made attempting to point out a relation ship between obvious psychological abnormalities and physiological problems related to Meniere s disease It is also shown that in many cases the course of the disease can be reversed through psychotherapy With the evidence presented it is obvious that the psychological implications of Meniere s disease should not be ignored

Physicians have long accepted the realization that patients with Meniere s disease are subject to anxiety and tension states and that this stress can aggravate the symptoms already present In the last two decades there has evolved an increasing awareness and knowledge of psychosomatic diseases Physicians have begun to look at the anxiety and stress present in Menière s disease as perhaps the precipitating factors in the disease rather than the natural consequences of this perplexing disorder

It is the purpose of this study to show the relationship between the first occurrence of the symptoms and the life stress situations that precede them as revealed by scientific research

Meniere s disease as a psychosomatic disorder

Although research that can indicate the presence of a psychosomatic illness is sparse studies have been done in the last 35 years that support the assumption that Meniere s disea has aspects of a psychosomatic disorder

Schilder (1933) stated that the vestibular a paratus is under the partial influence of the pe sonality and that dysfunction of the vestibul apparatus is very often the expression of tw conflicting psychic tendencies This dysfun tion is displayed in dizziness which occurs almost every neurosis and that this neurosis c cause organic changes in the vestibular sphe He wrote

> Dizziness is a danger in the sphere of the ego ar occurs when the ego cannot exercise its synthetic fun tion in the senses but it also occurs when conflicti motor and attitudinal impulses in connection wi desires and strivings cannot be united longer Dizzine is as important from the psychoanalytic point of vie as anxiety

However it was not until the early nineteer fifties that Fowler & Zeckel (1952) applied th above principles to Meniere s disease In thi article the authors undertook to define the pro cess by which Neurosis can cause organi changes in the vestibular sphere For the 195 research Fowler & Zeckel worked with 23 pa tients who had been diagnosed as having Me niere s disease Thirteen were male and al subjects were between the age of 24 and 60 All patients were given a battery of personalit tests These patients were studied before dur ing and after attacks It was found that

> a basic disorder of personality usually is presen in patients suffering from Menière s disease and tha the first attack and many subsequent attacks are

caused by vascular phenomenon in the labyrinth which were brought on by emotional stimuli so that Meniere's disease is primarily a psychosomatic disorder The upsets in autonomic control are apparently directly related to unusually violent stimulation to the psyche in a specific type of personality which has been undergoing stress over a period of time Occasionally the stress involves physical fatigue illness and surgical or dental operations but usually stress is emotional in origin

Fowler & Zeckel believed as others do that the pathology of the disease was labyrinthine hydrops and that changes in the blood supply to the inner ear were responsible The progress of the disease was noted in the following manner

1 Meniere's dis ase can be caused by psychogenic factors
2 The effect of the psychogenic factors seems to involve slowing and arresting of the small blood vessels within the labyrinth on an adrenergic pattern
3 Excessive lumping of red blood cells and early blood sludge in bulbar conjunctiva can be seen before and after attacks
4 It is sugge ted that these lumps of blood when caught in the smaller vessels of the labyrinth are instrumental in the production of the labyrinthine hydrops which has been found to be a characteristic pathology of the disease
5 Medical treatment and surgery will for the time being have to be continued in the case of patients with advanced Meniere's disease but in a large percentage of cases psychotherapy is necessary in addition to any of these forms of help
6 In early cases psychotherapy may reverse the course of the disease so that there are few if any residuals
7 Since there is much similarity in the significant traits in all these patients group psychotherapy treatment should be considered especially in clinics where individual therapy is too time consuming and costly

In a follow up article Fowler & Zeckel (1953) defined the personality profiles of the 23 patients used in the 1952 study and attempted to draw a more psychophysiological pattern for the disease All patients were given the Bernreuter Personality Inventory Picture Frustration Test by Rosenzweig the Jenkins Trait Study the Rorschack Test and the Thermatic Appreciation Test These tests plus interviews with the patients made it possible for the authors to divide the twenty three into five groups In each group one outstanding life situation seemed to be a common factor which immediately preceded an attack of vertigo The groups were

1 Personal Antagonisms Attacks of vertigo had a temporal connection with situations of antagonism to some individual or group important to the patient's welfare
2 Sexual Abstinence All males this group had abnormal avoidance of sex relations with a constant state of tension
3 Sexual Conflicts All women live in a continuous state of tension and agitation with conflicts centered around sexuality but involving other life stress events evoking stormy emotional reactions
4 Death All had experienced death of a near relative
5 Various Life Stresses

All of the above groups showed attacks of vertigo and later tinnitus and deafness that could definitely be related to a life stress situation Fowler & Zeckel believed that there was a fundamental predisposition but first attacks of vertigo as well as later ones tended to follow an explosive outburst of backing up of tension in an underlying intolerable situation The interference with circulation in the inner ear is explained by the authors as

1 Emotional tension and anger involve release of adrenalin or adrenalin like substances
2 Sympathetic stimulation and adrenalin have been found to produce classic slowing up of the peripheral circulation with narrowing of blood vessels In addition there is a lumping of blood in the bulbar conjunctiva which resembles a blood sludge
3 Patients with Meniere's disease exhibit excessive quantities of blood sludge plus slowing of circulation in their bulbar conjunctiva immediately before and after attacks
4 That the blood sludge composed of well formed clumps of blood are likely to stick and recur in injured areas
5 If the vascular pathology persists it can produce the labyrinthine hydrops characteristic of the disease
6 That the above are worthy of note as possible etiologic factors in Meniere's disease

Hinchcliffe (1967*a*) sought to pursue the idea of Meniere's disease as a psychosomatic disease further In his research he compared 20 persons with otosclerosis with 44 persons with Meniere's disease He discovered that emotional factors are involved much more frequently with

the onset of Menieres disease than with otosclerosis Out of 44 patients with Menieres disease 28 had emotional factors they related to the onset of the disease In the otosclerotic group only 2 out of 20 related emotional involvement to the onset of the disorder

Upon further research Hinchcliffe (1967 *b*) stated that he felt Menieres disease should be classified with the psychosomatoses In a study using the Minnesota Multiphasic Personality Inventory he found a prevalence of psychosomatic type personality profiles in people with Menieres disease compared with the control population His results showed 11 of such personality profiled out of 20 for the otosclerotics

Whenever the possibility of Menieres disease being a psychosomatic illness is discussed there are always divergant viewpoints One is that the psychological disturbances are secondary to the vestibular disorder (somatopsychic) and the one that has been discussed in this report that the psychological disturbances were primary to the vestibular disorder (psychosomatic) Results from studies in the last 35 years have revealed the significant occurrence of certain personality profiles and emotional disorders in those patients with Menieres disease when compared with a control population Evidence pointing to Menieres disease as a psychosomatic disorder is increasing Until such evidence can be disproved it is important that it not be ignored Perhaps for the time being it is most logical to view Menieres disease as a combination of the above two viewpoints that is

a group of somatopsychic effects associated with psychosomatic syndromes (Hinchcliffe 1967 *b*)

ZUSAMMENFASSUNG

Es ist medizinischen Sachverständigen seit vielen Jahren bekannt dass ungewohnliche Beängstigung und Gefuhlsspannung eine Folge der Menièreschen Krankheit sein können Es wird in dieser Abhandlung versucht die Moglichkeit zu veranschaulichen dass ein Zusammenhang zwischen den Symptomen der Menièreschen Krankheit und Gefuhlsspannungen besteht Wahrend der vergangenen 35 Jahre wurden mehrere vorsichtig regulierte Untersuchungen unternommen um die Verhaltnisse zwischen psychologischen Unregelmassigkeiten und den psychologischen der Menièreschen Krankheit verwandten Probleme deutlich klar zumachen Es wurde auch bewiesen dass eine Verbesserung der Krankheit durch Psychotherapie erfolgen kann Angesichts dieses Beweismaterials wird es klar dass die psychologische Verwicklung der Menièreschen Krankheit nicht unbeachtet bleiben darf

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EXPERIMENTAL POLLINOSIS

An Electron Microscopic Study

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Abstract The author has studied chronologically with the electron microscope the cellular alterations that follow the penetration of the pollen (*Betula alba*) into a sensitized mucosa The study has been done between one hour and twenty seven days A precise sequence of different cell waves" going from the eosinophil to the plasmocyte is described and as a conclusion a theory of the immunological autonomy of the nasal mucosa is proposed

What is the fate of the pollen grain when it happens to be inhaled by a iso sensitized mammal?

What happens then in this sensitized mucosa from the immuno-cytological point of view and finally how are such pneumallergens able to provoke a general and local hypersensitized state?

Among many questions that may be asked about pollinosis we have tried to answer the above ones It has been demonstrated that pollen grains were able to penetrate into the sensitized nasal mucosa (Stromme 1955 Chevance 1957) This histological finding raises new questions As a matter of fact the normal physiological activity of the upper respiratory ciliated epithelium is precisely to prevent such a penetration of inhaled particles into the mucosa

The combined actions of ciliary beating and of the mucous film in which the cilia are actively beating normally prevent the penetration of all insoluble particles of more than roughly three microns in diameter Obviously the size of most pollen grains ranges far above such dimensions

The experimental work was partly supported by a grant of the Fondation pour la Recherche Medicale française" 66 Bd Latour Maubourg Paris (VI)

MATERIAL AND METHODS

We have studied the fate of birch pollen grains (*Betula alba*) which were brought into contact with the sensitized nasal mucosa of guinea pigs (or rabbits) This study was carried out on a *chronological basis* the animals being sacrificed from 30 min to 27 days after the local anaphylactic shock Both light and electron microscopic techniques were used to follow the protracted course of the transformations that the pollen grain and the surrounding mucosa undergo

The birch pollen we have used was of two physical types either industrially dried pollen (Diephuis Laboratory Groningen The Netherlands) or in some cases fresh pollen grains prepared in our laboratory kept in the cold and used for experimental purposes within 2 or 3 weeks of harvesting Kept under such conditions the pollen is still fertile

No immunological differences were noted between the results caused by these two types of pollen It can be stated that the germinative part of the pollen has no direct influence on the

development of the allergic state and that only the proteic structure of the pollen content has a direct bearing on it

The sensitization of the animals (a total of 160 guinea pigs and 24 rabbits) was effected by both general injections and nasal instillations The animals received 5 intramuscular injections of a pollen extract combined with Freund adjuvant (0 5 ml each) The pollen extract was prepared by grinding 10 mg of pollen in 1 ml of distilled water The microscopic examination of the suspension shows that owing to the hardness of the pollen shell many grains appear intact but after 1 hour of contact the filtered suspension contained always more than 1 mg/ml of proteic nitrogen

The first four injections were given at 4 day intervals Between the second and third injection for 8 days the animals underwent instillation twice daily The same solution was used for nasal instillation as for injection except for the omission of the Freund adjuvant After a free interval of 15 to 18 days the animals received a fifth and last injection and two nasal instillations

Four days later each animal received an instillation of dry pollen and was sacrificed from 0 min to 27 days subsequently As controls unsensitized guinea pigs which had received only this last instillation were used

The electron microscopic study obviously requires a very precise technique in order to detect pollen grains on the small dissected fragments of mucosa To solve this problem the following method was elaborated By use of a trephin a small hole about 2 5 mm in diameter is made in the nasal bones about 2 cm from the tip of the nose and 5 mm from the ridge of the nose The underlying mucosa is opened with a fine thermoneedle so that no blood enters the nose Then at a known angle a fine Pasteur pipette is inserted into the hole and deposits the pollen grains at the contact of the mucosa of the septum without injuring it.

The bony defect made by drilling is always easily found even after 30 days survival (at present the longest period of study) and though by now filled with fibrotic repair tissue It is thus a simple matter to dissect at the correct location 2 5 mm of the nasal septum mucosa These samples are fixed by the usual technique (3 % glutaraldehyde 1 % osmium tetroxide) and then embedded in epoxy resin The resulting block is cut on an ultramicrotome at 2 μ and collected on slides in such a way that the cuttings are sufficiently thin to allow examination of the whole material under the phase-contrast microscope Under such conditions it has always been possible to detect the pollen grain in the mucosa due mainly to the shape of the pollen shell

The cuttings are transferred by mounting them with Araldite on a prepared block of epoxy resin these blocks can then be easily trimmed under the magnifying glass of the ultramicrotome

Chronological study of the interactions between the antigenic pollen and the cells of the nasal mucosa

The first stages of nasal anaphylactic shock have been analysed previously (Chevance 1957) and we have emphasized the importance of the destruction of the cilia during the first 30 min after pollen instillation The sequence of events may be summarized as follows

1 From the first to the tenth minute cessation of ciliary beating

2 From the tenth to the thirteenth minute destruction of the ciliary structures bulging and destruction of the epithelial cells and consequently penetration of the pollen grain into the mucosa and deeper into the tunica propria by progressive destruction of the cells on the way (Figs 1 2)

3 From the thirtieth minute to 48 hours surrounding of the antigen by polynuclear mainly eosinophil cells the antigen–antibody cytotoxic manifestations progress (Fig 3)

4 From the second to the tenth day the pollen is surrounded by wandering cells (mainly macrophages) These cells are submitted to cytotoxic effects owing to the steady extrusion

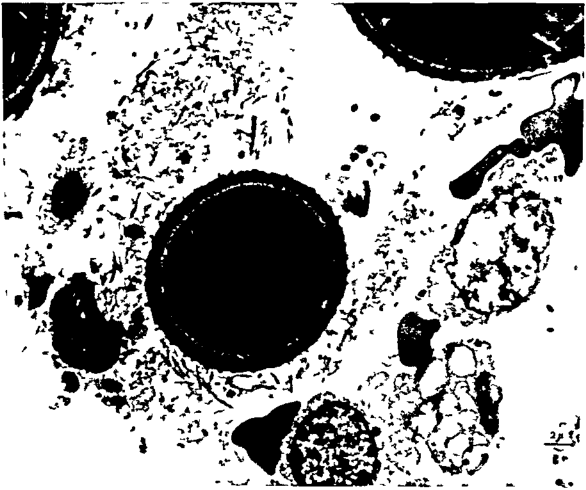


Fig 1 Penetration of the pollen grain into the mucosa

of the pollen antigen proteic content into the surrounding cellular atmosphere (Figs 4 5)

5 From the tenth to the fifteenth day the extrusion of the pollen content is slowly decreasing and consequently the acute phase of the antigen–antibody reaction comes to an end The macrophagic reaction around the antigen is still very strong For the first time cells appear whose cytoplasmic content displays an intense capacity for proteic elaboration (Fig 6)

6 From the fifteenth to the twenty seventh day the plasma cell reaction becomes evident the plasma cells appear in close contact with the macrophages and never with the pollen shell The cytoplasmic structures of these cells display morphological evidence of protein elaboration activity Simultaneously the gradual fragmentation of the empty pollen shell begins with the progressive lysis of both the intine and the exine

These results raise the question of

Local immunological activity of the upper respiratory mucosa

From the allergic and pharmacodynamic point of view this question is obviously of the utmost importance

If in the flat and seemingly uninteresting cellular structures of the nasal (and tracheal) mucosa it can be demonstrated that the





Fig 2 Splitting of the eosinophil cells in contact with the antigen

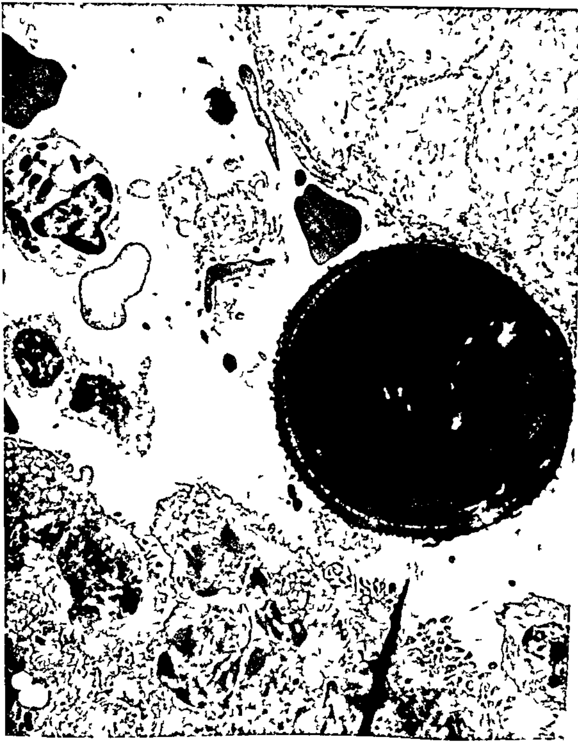


Fig 3 The antigen antibody cytotoxic effects after instillation



Fig ? Splitting of the eosinophil cells in contact with the antigen



Fig 5 Steady extrusion of the pollen content 5 days after instillation

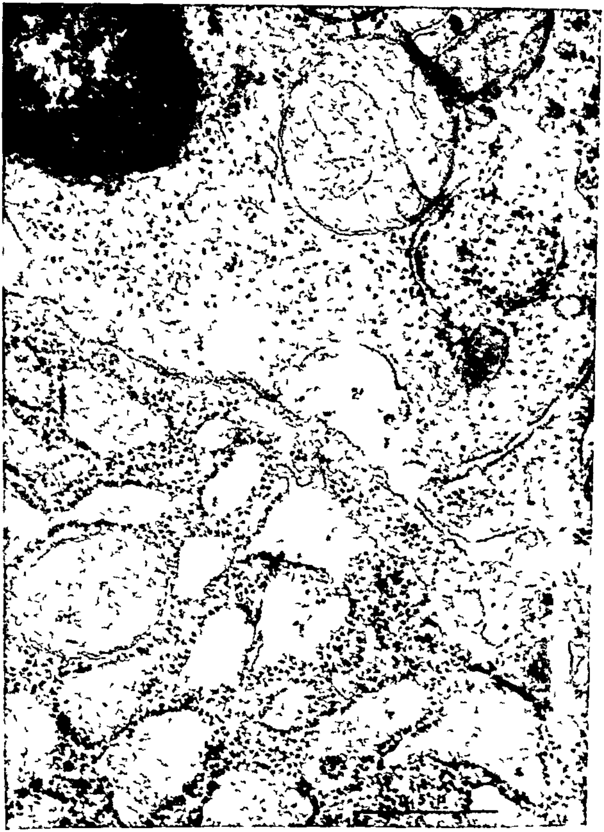


Fig 6 1? days after instillation Appearance of plasma cells



Fig 7 Plasma cells

The accuracy of cytologic diagnosis from smears of biopsy aspirate was judged in this study by comparing cytologic reports with the histologic findings in the subsequently extirpated nodes Of the 257 cases which were suitable for such analysis 17 (6 6) had received a false negative cytologic report from the first aspira tion biopsy (Table VI) The negative biopsy result may be explainable in two ways One is that at the time of needling the metastatic tumour growth involved only a minor area of the node and was missed by the needle Alter natively the metastatic growth could have occurred after the initial aspiration biopsy since the interval between this and extirpation of the node ranged from a few days up to 12 months Although repetition of the aspiration biopsy in clinically suspected but cytologically negative cases may be expected to reduce the number of false negative cytologic reports repeatedly negative findings should not unre servedly be considered to exclude malignancy If any clinical doubt persists the lymph node should be excised and histologically examined

The risk of false positive cytologic reports from lymph node aspirate must also be consid ered It is true that alien cells are readily recog nizable in aspirate from a structure clinically judged to be a lymph node But dermatogenic occlusion cysts and especially in the neck region congenital cleft cysts and carotid body tumours may sometimes be clinically mistaken for enlarged lymph nodes and aspirate from a dermatogenic occlusion cyst (epithelioma Mal herbe) or a congenital cleft cyst may contain squamous epithelium which is difficult or impossible to distinguish from metastasis of highly differentiated squamous cell carcinoma (Engzell & Zajicek 1970) Similarly aspirate from carotid body tumours may show epithelioid cells which to an inexpert eye could suggest metastasis from e g an adenocarcinoma of the thyroid (Engzell et al 1970) However if these difficulties are recognized the danger of false positive cytologic reports of metastatic carcinoma in lymph nodes of the neck may be disregarded

The distribution of the primary tumours which gave rise to the cervical lymph node metastases was also investigated For this purpose we reviewed the records of the 1 101 consecutively seen patients in whom lymph node metastasis was cytologically diagnosed by aspira tion biopsy

In a number of reports in the literature the sites of such primary tumours have been clas sified according to whether they were above or below the clavicle Comess et al (1957) investi gated 1 189 consecutive cases of cervical lymph node metastasis seen at the Mayo Clinic The primary tumour was above clavicular level in 77 4% and below the clavicle in 17 2 while in 5 4 the primary tumour site was never dis covered Skandalakis et al (1960) found 85 4 of 900 primary tumours above the clavicle and 14 6% below, and Skolnik et al (1965) reported respective frequencies of 80 7 and 19 3 % of 789 tumours

Slaughter et al (1956) studied 1 216 patients who were admitted to hospital because of a neck mass or for conditions in which welling of the neck was a dominant feature They found metastatic tumours in 413 patients 79 4 of whom had a supraclavicular and 20 6 an infraclavicular primary tumour In Ey s (1963) study of 925 patients who were operated on for enlarged cervical nodes 373 had metastatic carcinoma The primary site was supraclavicular in 92 5 infraclavicular in 2 7% and unknown in 4 8 Lennert (1963) reported 123 histologi cally studied cases with a supraclavicular in fraclavicular primary tumour ratio of 68 3 31 7

Hendrick (1952) found among 1 003 con secutively examined patients with various cervica tumours only 21 carcinoma metastases fron tumours below the clavicle

In the mentioned repo[illegible] therefore th primary tumours were located in the head anc neck region in 68 3 to 92 5 of the patient: with metastases in cervical lymph nodes

In the 1 101 patients of the present series the distribution of the primary tumours differed to some extent from the figures given in the literature in that supraclavicular and in fraclavicular sites showed a similar frequency

(Table I) The main reason for the discrepancy may have been differing composition of the clinical series due in turn to the diagnostic method used In most of the series in the literature the diagnosis was made after surgery whereas we routinely used aspiration biopsy which causes a minimum of discomfort to the patients and can be used on widest indication

Many writers have discussed the question of occult primary tumours in patients with cervical lymph node metastasis Hendrick (1967) stated that in 197 (36 2) of 544 patients the site of the primary tumour was not revealed by the initial thorough physical examination Further investigations over some months led to detection of supraclavicular primary tumours in 127 patients (83 1) and infraclavicular primary tumours in 21 patients (16 9) The tumour site remained unknown in 49 patients (9 0 of the total series)

Of the 1 189 patients studied by Comess et al (1957) 50 (4 2) died without evidence of a primary neoplasm

France & Lucas (1963) studied 43 patients in whom no primary tumour was found within 2 months of a diagnosis of cervical metastases In 9 of them the occult tumour was discovered before death The remaining 34 patients of this group represented 6 3 of the total number of metastatic cervical node carcinomas which France & Lucas had encountered

A series of 127 patients with unknown primary tumours despite thorough clinical investigation after detection of metastatic carcinoma in cervical nodes was reported by Jesse & Neff (1966) The total number of patients with such metastases was not stated In 48 of the 127 patients the primary tumour was detected before death It was above clavicular level in 29 patients (60 4) and below the clavicle in 19

In collected cases from the literature Cachin et al (1969) found 817 with cervical lymph node metastases and unknown primary tumours The primary lesions were later detected in 317 patients and were sited as follows above the clavicle in 72 6 intrabronchially in 10 4 in the oesophagus in 6 9 and in the thoracic or abdominal regions in 10 1 The writers did not specify how many of the tumours were diagnosed before death

Thus in the literature here reviewed 4 2 to 9 of patients with lymph node metastasis of the neck died without diagnosis of the primary tumour When occult primary tumours were revealed 60 4 to 83 1 of them were located above the clavicle

Martin & Morfit (1944) observed 218 patients in whom cervical lymph node metastasis was the first sign of cancer In 71 of them no primary tumour was found within a month The occult tumours were later discovered in 16 patients but remained unknown in 55

In the present series of 1 101 patients 133 (12 1°) of the primary tumours were still undetected 1 month after needling of the cervical nodes had revealed metastasis Forty four of these primaries were found ante mortem 21 above and 23 below the clavicle (Table II) Seventy nine of the patients (7 2) died before diagnosis of the primary tumour which however was revealed by necropsy in 39 cases and was subclavicular in 37

In the follow up study the five year survival rate was low (11 5) among the patients with undiscovered primary tumours No improvement of this figure is to be expected unless diagnostic refinements speed the detection of primary tumours

One of the major advantages of aspiration biopsy as pointed out by Martin & Ellis (1934) is that it saves time in arriving at the definite diagnosis so that the nature and order of therapy can be immediately planned This is especially true when the source of lymph node metastasis is known Otherwise a cytologic diagnosis of metastatic carcinoma initiates or stimulates a search for the primary tumour In 34 7 of the patients in the present study the primary tumour had not been microscopically diagnosed at the time of the cytologic examination and in 12 1 it was still unknown 1 month later To what extent refinements of cytologic diagnosis in carcinomatous metastases of unknown source might influence the cli

management and survival cannot be judged at the present time However much time-consuming clinical work might sometimes be avoided if the cytologist when reporting carcinoma in lymph nodes could give the clinician some indication of the primary tumour site This aspect of the problem will be dealt with in future work

ZUSAMMENFASSUNG

Durch zytologische Untersuchungen von Ausstrich praparaten nach Punktionsbiopsien mit feiner Nadel wurden in der Zeit von 1955 bis 1966 bei 1 101 Patienten Karzinommetastasen in Halslymphknoten festgestellt Die Primartumoren wurden bei *522 Patienten (47 4)* oberhalb der Clavicula und bei 529 Patienten (48 0) unterhalb der Clavicula gefunden Bei 50 Patienten (4 5 %) verblieben die Primartumoren unbekannt.

Die zytologische Diagnose einer Metastase war in 382 Fallen (34 7 %) die erste Bestatigung einer vor handenen Malignitat In 133 dieser Falle hatte man innerhalb eines Monats nach der Punktion keinen Primartumor gefunden Der Primartumor war auch zum Zeitpunkt des Todes bei 79 Patienten (7.2) noch unbekannt

Eine funfjahrige follow up Untersuchung von 656 Patienten zeigte dass die Überlebensrate erhebliche von der Lage des Primärtumors abhangige Variationen aufwies Die totale funfjahrige Überlebensrate wurde auf 25 2 berechnet

Zytologische und histologische Untersuchungen derselben Lymphknoten konnten in 257 Fallen miteinan der verglichen werden. In 17 Fallen (6 6 %) wurde eine falsch negative zytologische Diagnose gestellt

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TREATMENT OF ACUTE MAXILLARY SINUSITIS

II *A Comparison of Five Further Methods*

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Abstract In a previous paper four different methods of treatment for acute maxillary sinusitis with secretion were compared with the aid of radiological registration of the sinus state In the present investigation five further methods are compared The results of the treatment by irrigation only or by high doses of penicillin V+nasal decongestant combined with irrigation or oxyphenbutazone or by methacycline+nasal decongestant were mutually similar and better than the results of treatment with penicillin V+nasal decongestant The bacteriological findings were in agreement with previous investigations demonstrating a high frequency of sterile secretions and of penicillin resistant bacteria and a predominance of Haemophilus influenzae in young individuals

Conventional treatment of acute maxillary sinusitis with irrigation is considered unpleasant by many patients and requires several consultations Since most of these patients belong to the productive members of society the illness means economical consequences for the patient as well as for society Thus it seems justified to search for alternative treatment where irrigation can be substituted by different equally effective measures requiring fewer consultations

In a previous paper (Axelsson et al , 1970 *b*) treatment with nasal decongestant only was compared with nasal decongestant combined with irrigation or with 0 4 g penicillin V three times daily or with 0 5 g lincomycin three times daily The results of the three combined treatments were similar and significantly better than treatment with nasal decongestant only

In the previous investigation the penicillin dose was fairly low By increasing the penicillin dose an improved therapeutic effect has lately been achieved in many infectious diseases Thus it was decided to compare the effect of high doses of penicillin solely with the same doses of penicillin combined with irrigation Further, it has recently been demonstrated that serum levels of penicillin V increase after simultaneously given oxyphenbutazone (Legler & Bracharz 1968 Frisk & Tunevall 1969) This then would offer advantages in two ways i e the antiphlogistic action of oxyphenbutazone and the increased serum levels of penicillin It was further considered to be of interest to compare these modes of treatment with irrigation only and with a broad spectrum antibiotic such as methacycline

MATERIAL AND METHODS

Patients attending the E N T department with suspected sinusitis were referred for radiological examination The method for the demonstration of sinusitis radiologically has been presented previously (Axelsson et al 1970 *a* Chidekel et al 1970) and has a documented high confidence concerning the demonstration of secretion in the maxillary sinus

The present investigation includes only patients with acute maxillary sinusitis with secre

Table I *Present material Number of patients and maxillary sinuses*

	Irrigation only	Penicillin + nasal decongestant	Penicillin + nasal decongestant + irrigation	Penicillin + nasal decongestant + oxyphenbutazone	Methacycline + nasal decongestant	Total
Women	26	31	31	32	27	147
Men	24	19	18	18	23	10[illegible]
Total	50	50	49	50	50	249
Mean age years	35	34	36	32	32	34
Sinuses analysed initially	74	77	74	78	81	384
Sinuses analysed after 5 days	74	76	72	77	80	379
Sinuses analysed after 10 days	74	71	68	72	80	365
Sinuses analysed after 15 days	71	72	68	77	81	369
One sinus affected	21	17	20	18	16	92
Both sinuses affected	21	22	17	23	28	111
Both maxillary and frontal sinuses affected	8	11	12	9	6	46
Not completely opaque sinuses	58	61	57	65	63	304
Completely opaque sinuses	16	16	17	13	18	80

tion The material contains 249 patients with 384 diseased sinuses In 46 of the patients 66 frontal sinuses were also affected but these may only have influenced the subjective evaluation of the clinical course In all 80 completely opaque maxillary sinuses a single diagnostic irrigation was performed at the first examination to demonstrate secretion Patients with an allergic history and patients where treatment had been initiated elsewhere were not included in the investigation The patients were randomly distributed in the five treatment groups i e every fifth patient received the treatment of group A etc

In 176 patients (71) nasal smears were obtained for bacteriological examination Secretion obtained from the diseased sinus at irrigation was also examined bacteriologically

The method of treatment for each group was as follows

A *Irrigation only* Patients were irrigated every second day until the lavage content was clear

B Penicillin V + nasal decongestant. Patients were treated orally with tablets of phenoxymethyl penicillin (Meropenin® Kabi) 1 2 g three times daily for 10 days and oxymetazolinechloride (Nesenl® Draco) three drops in each nostril three times daily

C Penicillin V + nasal decongestant + irrigation The patients in this group received the treatment of group A and of group B

D Penicillin V + nasal decongestant + Oxyphenbutazone (Tanderil® Geigy) The patients in this group received the treatment of group B and additionally tablets of oxyphenbutazone (Tanderil®) 0 2 g three times daily for three *days then further 0 1 g three times daily for* seven days

E Methacycline + nasal decongestant The patients were treated perorally with tablets of methacycline (Rondomycin® Roerig) 0 3 g twice daily

The radiological examinations of groups A and B were always made before the irrigation to reduce the risk of mistaking the irrigation liquid for sinus secretion All patients returned as close to the fifth tenth and fifteenth day of treatment as possible for radiological examination and subjective evaluation of the treatment

	Mean number of points/sinus			
	Before treatment	After 5 days	After 10 days	After 15 days
Irrigation only	4 04	3 08	2 03	1 32
Penicillin + nasal decongestant	3 82	3 30	2 54	2 01
Penicillin + nasal decongestant + irrigation	4 10	3 04	1 82	1 16
Penicillin + nasal decongestant + oxyphenbutazone	3 74	2 36	1 58	1 03
Methacycline + nasal decongestant	3 83	3 15	2 31	1 33

The radiological state of each affected sinus was then analysed according to the 6 point scale below and the improvement or the deterioration recorded In all healing was analysed in 379 maxillary sinuses on the fifth day of treatment in 365 on the tenth day and in 369 on the fifteenth day (Table I) The radiological state of the sinuses was classified at each examination as follows

Mucous membrane thickening ≤ 6 mm	1 point
Mucous membrane thickening > 6 mm	2 points
Secretion	2 points
Mucous membrane thickening ≤ 6 mm + secretion	3 points
Mucous membrane thickening > 6 mm + secretion	4 points
Complete opacity	6 points

The material was treated statistically according to the method of one way or two-way analysis of variance and simple linear regression The level of significance used in all tests was 95%

RESULTS

Before treatment there was no statistical difference in the radiological state between the five groups (Table II) Further there was no great difference between the groups initially concerning the number of unilaterally and bilaterally affected sinuses in each group neither between the number of complete and not complete opaque sinuses nor concerning the number of affected frontal sinuses (Table I) There was no statistical difference between the groups concerning mean age and sex All treatment groups demonstrated a progressive subjective and radiological improvement after 5 10 and 15 days (Table II Fig 1)

The following statistical differences could be demonstrated during the treatment In the total material unilaterally affected sinuses healed faster than bilaterally affected sinuses

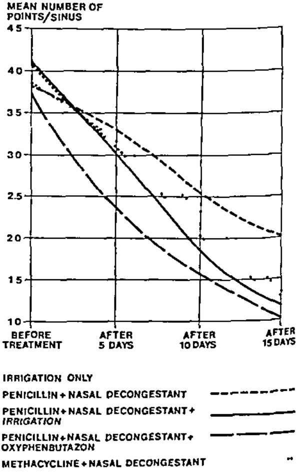


Fig 1

Table III *Side effects*

	Irrigation only	Peni illin + nasal decongestant	Penicillin + nasal decongestant + irrigation	Penicillin + nasal decongestant + oxyphenbu tazone	Methacycline + nasal decongestant
Nausea and/or vomiting	0	2 (1)	2	1	2
Epigastralgia	0	1		3 (1)	
Loose bowel movements	0	12 (4)	5	13 (3)	3
Abdominal pain	0	2	1 (1)	1	1
Fatigue	0	6	8	7	3
Stomatitis and exanthema	0	1	1	1 (1)	0
Pain after irrigation and/or epistaxis	3	1	1	0	0
Number of patients	3	18	17	21	6

igures in brackets indicate the number of patients with interrupted treatment owing to side effects

The radiological state in patients treated with rrigation only with penicillin + nasal deconge tant + irrigation with penicillin + nasal decon- estant + oxyphenbutazone and with methacyc ne + nasal decongestant were mutually similar ter 5 10 and 15 days Further these four oups were more improved than patients treated with penicillin + nasal decongestant after 15 days The last mentioned group was less improved than the group treated with penicillin + nasal decongestant + oxyphenbutazone after 5 and 10 days as well

Further certain tendencies could be discerned without amounting to the level of statistically significant difference (*a*) Patients treated with penicillin + nasal decongestant + oxyphenbuta zone were more improved than the other groups after 5 days (*b*) Patients treated with penicil lin + nasal decongestant considered themselves less improved than the other groups after 15 days

The side effects are noted in Table III In general they were mild However in eleven cases the side effects were so severe that the treatment was interrupted

The main part of the bacteriological exam ination will be published elsewhere (Axelsson & Brorson 1971) Some of the results are given in Tables IV and V Bacteria were demonstrated in the maxillary sinuses in 78% and sterile secretion in 22% The most common pathogenic bacteria were pneumococci (22) followed by Haemophilus influenzae (12%) and Staphylococcus aureus (11%) Haemophilus influenzae was considerably more frequently found in young than in older individuals Further the bacteriological findings were analysed in those maxillary sinuses which deteriorated during treatment and in the

able IV *Bacteriological findings in sinus secretion*

Whole material A total number of 293 findings from 244 maxillary sinuses in 176 patients
Sinuses curing least (deteriorated or unimproved) 63 findings from 54 maxillary sinuses in 38 patients

	A		B	
	Number	%	Number	
rowth	64	22	19	30
h albus	26	9	4	6
h aureus	32	11	7	11
ia hemolytic p	25	9	4	6
hemolytic p	6	2	1	2
robic strep	3	1	1	1 5
mococci	64	22	6	10
ococci	3	1	0	0
fluenzae	36	12	14	22
ra influenzae	3	1	0	0
eria	10	3	1	1 5
ic theroids	3	1	3	5
robic theroids	1	0	0	0
rms	4	1	1	1 5
is	2	1	1	1 5
obic Gram ods	11	4	1	1 5
	293	100	63	100

meningococci or gonococci

Table V *The radiological course in sinuses with bacteria resistant to penicillin before treatment*

Mode of treatment	No of patients	No of sinuses	Mean no of improvements points (after 15 days treatment)	Mean no of improvement points Whole group (after 15 days treatment)
Penicillin + nasal decongestant	8	15	1 13	1 81
Penicillin + nasal decongestant + irrigation	12	18	2 88	2 94
Penicillin + nasal decongestant + oxyphenbutazone	3	4	3 17	2 71

severe diseased sinuses which did not improve during the treatment (Table IV) Bacteria were found in 70% and sterile secretion in 30% Additionally the radiological course was examined in those maxillary sinuses where bacteria were demonstrated to be resistant to the antibiotic given (Table V) In the group treated with methacycline there was only 1 patient with resistant bacteria (omitted in the Table) In the group treated with penicillin + nasal decongestant sinuses with penicillin resistant bacteria were cured more slowly than on average

DISCUSSION

… are very few published investigations comparing different methods of treatment for acute maxillary sinusitis This is probably due to the lack of reliable methods for measuring cure in this disease Neither the number of irrigations needed (Kortekangas 1963) nor the determination of the concentration of an antibiotic in sinus secretion (Lundberg et al 1968) gives an appropriate answer to the question of healing The present method measuring the radiological improvement (Axelsson et al, 1970 *a* Chidekel et al 1970) is intended for comparison between different modes of treatment and has the advantage of being applicable in both irrigation and non irrigation groups and presumably gives a more accurate indication of the clinical course than the determination of antibiotic concentration

Mucous membrane thickening in the maxillary sinus not only indicates infection but also allergy or vasomotor influence Patients with a history of allergy or vasomotor rhinitis were excluded from the present investigation This does not necessarily mean that it was possible to exclude every such case by means of the anamnesis and the clinical examination but from the statistical viewpoint it can be presumed that any allergic or vasomotor patient is randomly distributed in the different treatment groups The present investigation contains only maxillary sinuses with secretion Cases with mucous membrane thickening without secretion were excluded to reduce the number of allergic and vasomotor cases Further the occurrence of secretion reflects a more severely impaired drainage in infectious sinusitis and consequently a more severe disease In the present investigation the term sinusitis is used when secretion is demonstrated in the sinus regardless of the result of the bacteriological examination

It would have been an advantage if the different methods for treatment could have been compared with an untreated control group For obvious reasons this is difficult to accomplish Instead the present and the previous investigations compare mere drainage measures with antibacterial treatments and combinations of T… sinuses were … demonstrate sec… may have influ… …e non irrigation … the initial … …nicillin + … in + … n…

nasal decongestant + oxyphenbutazone group and 18 in the methacycline + nasal decongestant group The number of irrigations needed until the lavage content was clear was 4 04 times in the irrigation group and 3 64 times in the penicillin + nasal decongestant + irrigation group There is no statistical difference between these two figures

As previously pointed out only maxillary sinuses with secretion were included in the investigation This indicates an impaired drainage from the sinus but not necessarily a more severe infection from the bacteriological viewpoint This is confirmed by the finding that no growth was demonstrated in 22% of the diseased sinuses and that many apathogenic bacteria were found (Table IV) The distribution of the pathogenic bacteria is in good agreement with the findings of previous authors (Bjorkwall 1950 Kortekangas 1963 Lystad et al 1964) The bacteriological findings in those sinuses which deteriorated or did not improve during treatment demonstrate a high frequency of Haemophilus influenzae and a low frequency of pneumococci This indicates that Haemophilus influenzae causes a more longstanding sinusitis than average Otherwise the findings for the sinuses which healed least are in agreement with the whole material This indicates the importance of factors other than the bacteriological for the clinical course of the acute maxillary sinusitis presumably the function of the sinus ostium This is confirmed by the findings of Drettner (1965) who demonstrated an impaired permeability of the maxillary ostium in about 50 of cases with acute sinusitis

The radiological course in sinuses with bacteria resistant to the antibiotic given was investigated (Table V) The incidence risk of encountering bacteria resistant to penicillin was about 16 It can be seen that there is a difference between the three penicillin groups In sinuses with resistant bacteria treated with penicillin + nasal decongestant but without additional measures the improvement is considerably less than the improvement of the whole group The unsatisfactory results obtained with penicillin + nasal decongestant indicate the importance of additional treatment such as irrigation and/or oxyphenbutazone which gives at least an average improvement when compared with the whole treatment group even in cases with penicillin resistant bacteria The high frequency of penicillin resistant bacteria the risk of allergic reactions and the inferior results with penicillin without additional treatment makes it doubtful if penicillin is the proper antibiotic for the treatment of acute maxillary sinusitis

The treatment with penicillin + nasal decongestant was less effective than the other modes of treatment in spite of a high dosage It is interesting to note that this high dose was not more effective than the lower dose used in the previous investigation A probable explanation for this can be that the patients suffered from considerably more side effects with the higher dose and thus might not have taken the ordered dose throughout

The irrigation group may be considered as a control group aimed for comparison of a mere drainage method with alternative treatment The result of this treatment is better than penicillin + nasal decongestant and equally effective as penicillin + nasal decongestant combined with irrigation Thus the addition of high doses of penicillin does not offer any advantage if the decision is made to treat the sinusitis by irrigation This opinion is also held by Kortekangas (1963) However if penicillin is combined with nasal decongestant and oxyphenbutazone similar results are achieved as when penicillin is combined with irrigation or with irrigation only The addition of oxyphenbutazone thus offers the advantage of omitting irrigation in the treatment The effect of oxyphenbutazone can be due to different modes of action i e the antiphlogistic effect and/or the increased serum levels of penicillin (Legler & Bracharz 1968 Frisk & Tunevall 1969) Unfortunately treatment with penicillin + nasal decongestant + oxyphenbutazone was accompanied by the highest frequency of side effects mainly due to the effect of the high penicillin dose on the in

testinal bacterial flora but also partly due to the irritating effect of oxyphenbutazone on the gastric mucosa The treatment with methacycline + nasal decongestant was better than treatment with penicillin + nasal decongestant and equally effective as the other modes of treatment Further methacycline has the advantage of a low frequency of side effects

The patients subjective evaluation of the clinical course agreed well with the radiological state during the treatment

As a conclusion of the previous and the present investigation the following remarks can be made Of the simple drainage measures irrigation is more effective than nasal decongestant The combination of both measures does not offer any advantage over irrigation only Consequently nasal decongestants can be omitted when treating with irrigation Penicillin is probably not the most appropriate antibiotic for the treatment of acute maxillary sinusitis To achieve good therapeutic results without adding irrigation, penicillin has to be combined with oxyphenbutazone and the penicillin dose evidently must not be too high since side effects then increase considerably The serum levels of penicillin increase with oxyphenbutazone and it would have been interesting to compare the result of the present investigation with a group treated with oxyphenbutazone combined with a considerably lower penicillin-dose to decrease the side effects Since irrigation is considered unpleasant by many patients and requires several consultations it has been the aim to search for methods in which the irrigation can be omitted without negatively influencing the results This was achieved by two modes of treatment penicillin + nasal decongestant combined with oxyphenbutazone and by the treatment with methacycline + nasal decongestant The latter treatment has the advantage of a low frequency of side effects and of simple dosage twice daily

In a future investigation the promising results of broad spectrum antibiotics will be further studied in the search for the optimal treatment of acute maxillary sinusitis

ZUSAMMENFASSUNG

In einer fruheren Mitteilung wurden vier verschiedene Behandlungsmethoden der akuten Maxillarsinusitis mit Hilfe von radiologischer Registrierung des Sinusbefundes verglichen In der vorliegenden Untersuchung *werden fünf* weitere *Methoden* miteinander verglichen Die Resultate der Behandlung mit Spulung allein oder mit hohen Dosen von Penicillin V + Nasentropfen kombiniert mit Spulung oder mit Oxyphenbutazon *oder mit Methacycline* + Nasentropfen waren einander ahnlich und besser als das Resultat der Behandlung mit Penicillin V + Nasentropfen Die bakteriologischen Befunde waren in guter Übereinstimmung mit fruheren *Untersu hungen* indem sei eine hohe Frequenz von Penicillin resistenten Bakterien sowie eine Dominanz von Haemophilus Influenzae bei jungen Individen zeigten

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VESTIBULAR RECRUITMENT IN MENIÈRE S DISEASE

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Abstract Patients with unilateral Menieres disease were investigated with regard to vestibular recruitment. They were accelerated clockwise and counter clockwise respectively at 1 2 4 and 8 /sec² After each acceleration there was an interval of about 6 minutes at constant velocity Latency times crescendo times and after discharges were studied The results were compared with those obtained for a control material consisting of healthy persons On acceleration to the affected ear the latency times for the patients with Menieres disease were longer than on acceleration to the healthy ear However the difference was not statistically significant. On the other hand compared with the latency times of the control material they were significantly longer statistically on acceleration at 1 and 4 /sec² When the acceleration strength to the affected ear was increased from 1 to 8 /sec² the latency time was decreased to a significantly greater extent than for the corresponding increase in acceleration in the control material The crescendo times for the patients with Menieres disease were the same on acceleration to the affected and to the healthy ear and were throughout slightly longer than in the control material The after-discharges were of the same length on acceleration both to the healthy and to the affected ear At 1 2 and 4 /sec² they were significantly shorter statistically than the after discharges of the control material On increasing the acceleration strength from 1 to 8 /sec² the after discharges were prolonged to a significantly greater extent statistically than in the control material The investigations have shown that patients with unilateral Menière s disease display positive vestibular recruitment with regard to both latency times and after discharges

Menière s disease is usually associated with impairment of hearing caused by injury to the sensory cells in the organ of Corti A characteristic feature of such an auditory injury is the occurrence of recruitment which implies an abnormally rapid increase in auditory response when the acoustic stimulus is increased above the raised threshold (Dix et al 1948) It is well known that the vestibular part of the inner ear is also frequently injured in Meniere s disease and one can ask oneself whether this part, on the analogy of a cochlear injury also exhibits recruitment If so it should be possible to establish a raised vestibular stimulus threshold and an abnormally strong intensification of response on stimulation with successively increased stimuli above this threshold

Rotatory investigations of patients with Meniere s disease have been previously conducted by the cupulometric method This means that the investigated subject is rotated at different constant velocities between about 2° and 70 / sec and is subsequently brought to a standstill within ¹/₂–1 sec after which the duration of nystagmus is measured and at the same time duration of subjective dizziness is often assessed Consequently a patient with vestibular sensory cell injury should have a cupulometric curve that on the one hand starts with a higher initial velocity and on the other has a steeper course than that described for healthy persons by van Egmond et al (1948) and Aschan et al (1952) The results obtained vary considerably Some authors have reported a lowered stimulus threshold (Portmann & Boussens 1961) whereas other authors have shown a raised stimulus threshold Most of these cupulograms have either the same or a less steep slope than that of the cupulograms of healthy persons and this appears rather to contradict

vestibular recruitment (Milojevic & Subotic 1963) Still other authors have stated that patients with Meniere s disease have quite normal cupulograms (van der Vis 1957)

At the same tim- rotatory investigations of another type have been made namely, so-called threshold measurements It has been found that patients with Meniere s disease have a raised threshold for the nystagmus reflex on both clockwise and counterclockwise acceleration (Montandon & Russbach 1956 Russbach & Tarab 1957, Montandon 1961)

The previous investigations which were made with rotatory stimuli were not designed to deal with the problem whether patients with Meniere s disease have recruitment Hence a method has been developed with accurately graded stepwise increased rotatory accelerations The following nystagmus components have been studied each one separately in healthy subjects latency time crescendo time and after discharge (Fluur & Mendel 1966 1969 *a* *b* 1970 Fluur et al 1967) In view of the fact that in these investigations each of the nystagmus components was studied separately it has been consid red important to study a control material where all the components were investigated at the same time in order to obtain exactly similar investigational conditions as in the subsequent investigation of patients with Meniere s disease

MATERIAL

As a control material 22 persons were investigated They consisted partly of the personnel at the Department of Otolaryngology and partly of patients with diseases that could not be regarded as having any effect on an investigation such as this The Meniere s material contained 22 persons between the ages of 26 and 64 years and the age distribution was about the same as in the control material They all had Meniere s disease and a typical history of dizziness tinnitus and impairment of hearing The impairment of hearing was either on the left side (14 cases) or on the right side (8 cases) Some subjects however had slight impairment of hearing also in the other ear but as a rule the impairment had then the character of presbyacusis and affected the higher frequency range Fowler s test was performed in each case to confirm that the impairment of hearing in the so called affected ear was cochlear and the test was positive throughout All the subjects were carefully examined in order to exclude neurological disease The measurements were made during periods when the patients had no dizziness and a control was also made to ensure that no pharmaceuticals were taken

Apparatus and mode of stimulation

The subject sat in an electrically driven rotation chair with the head secured in a holder and inclined 30° forward All the experimental arrangements and recording conditions were the same as described in a previous investigation by Fluur & Mendel (1966) The stimulation intensities and times were as follows

1°/sec for 40 seconds
2°/sec for 40 seconds
4°/sec- for 30 seconds
8°/sec for 20 seconds

These acceleration times can be considered sufficient to elicit a fully develop-d vestibulo-ocular reflex (Fluur & Mendel 1970) All the examinations were made at about the same time of day

Calculations

All the nystagmograms in the two materials were assessed in the same way which implies the measurement of latency time crescendo time and after-discharge If a reliable measurement could not be made this was indicated by a negative sign (−) in the tables

For the control material the arithmetic mean values and standard deviations were calculated for the different acceleration strengths clockwise and counterclockwise and it was calculated how each individual s latency time changed

stimulation intensity was increased from c to 8 /sec The mean values and stand eviations were calculated also for these es

r the latency times of the patients with ere s disease the following calculations made The arithmetic mean values and lard deviations were calculated for the dif it acceleration strengths to the affected and healthy ear respectively so as to facilitate parisons Then the change in each indi al s latency time was calculated when the ulation intensity was increased from 1°/ to 8 /sec and the mean values for these ages were calculated

or crescendo times and after discharges the ie calculations were made

inally comparisons were made and sta ically analysed

RESULTS

e results are listed in the tables These show latency times (Table I) crescendo times able II) and after discharges (Table III) for e control material and the material with Me ere s disease A common feature in both aterials is the wide variations between dif rent persons and for some measurements also ie values for the same person can vary very onsiderably Therefore for assessing the re alts the mean values have been calculated and tatistical analyses performed

CONTROL MATERIAL

Latency times

The latency times varied particularly widely for the weakest acceleration strength 1 /sec The mean values for clockwise acceleration were at 1 /sec 8 9 sec and at 2° 4 and 8°/sec the latency times were increasingly shorter 6 0 sec 2 7 sec and 2 0 sec By increasing the ac celeration strength from 1 /sec to 8 /sec the latency time was decreased by 6 9 sec For counterclockwise acceleration the latency times were throughout shorter Thus the values were 5 8 sec at 1°/sec 2 9 sec at 2 /sec 2 0 sec at 4°/sec and 1 5 sec at 8 /sec Here the latency time was decreased by 4 3 sec when the acceleration strength increased from 1° to 8 /sec In order to obtain a better survey of the results the mean values for the latency times for stimulation in both directions were taken together We then obtained at 1°/sec 7 4 sec at 2 /sec 4 5 sec at 4°/sec 2 4 sec and at 8 /sec 1 8 sec

Crescendo times

The crescendo times also varied widely on comparing different individuals (Table II) but the relative variations were less than those for the latency times At 1°/sec clockwise accele ration a crescendo time of 21 1 sec was obtained at 2 /sec 19 3 sec at 4°/sec 12 1 sec and at 8°/sec 9 4 sec (Fig 2) An increase in acceleration strength from 1°/sec to 8°/sec resulted in a decrease in crescendo time of 11 7 sec Counterclockwise acceleration gave throughout slightly lower values namely 14 7 12 6 11 0 and 7 6 sec respectively The de crease was 7 1 sec when the acceleration was increased from 1° to 8 /sec The combined m an values for both directions were at 1 / sec 17 9 sec at 2 /sec 16 5 sec at 4°/sec 11 6 sec and at 8 /sec 8 5 sec

After discharges

The after discharges increased when the ac celeration strength was increased (Table III) At 1 /sec clockwise acceleration the mean value was 10 4 sec and at the other accelera tion strengths 2 /sec 4 /sec and 8 /sec 16 8 sec 23 4 sec and 23 2 sec respectively were obtained This means that the after-dis charge increased by 12 8 sec when the accelera tion strength was increased from 1 /sec to 8°/sec The corresponding counterclockwise accelerations resulted in afterdischarges of 19 0 sec 23 8 sec 25 1 sec and 27 5 sec respec tively i e a prolongation of 8 5 sec when the acceleration strength was increased from 1° to 8 /sec The mean values for both directions of acceleration were at 1°/sec 14 7 sec at

vestibular recruitment (Milojevic & Subotic 1963) Still other authors have stated that patients with Meniere s disease have quite normal cupulograms (van der Vis 1957)

At the same time rotatory investigations of another type have been made namely so-called threshold measurements" It has been found that patients with Meniere s disease have a raised threshold for the nystagmus reflex on both clockwise and counterclockwise acceleration (Montandon & Russbach 1956, Russbach & Tarab 1957 Montandon 1961)

The previous investigations which were made with rotatory stimuli were not designed to deal with the problem whether patients with Meniere s disease have recruitment Hence a method has been developed with accurately graded stepwise increased rotatory accelerations The following nystagmus components have been studied each one separately in healthy subjects latency time crescendo time and after discharge (Fluur & Mendel 1966 1969 *a* *b* 1970 Fluur et al 1967) In view of the fact that in these investigations each of the nystagmus components was studied separately it has been considered important to study a [illegible] material where all the components were [illegible] at the same time in order to obtain exactly similar investigational conditions as in the subsequent investigation of patients with Meniere s disease

MATERIAL

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For the control material the arithmetic mean values and standard deviations were calculated for the different acceleration strengths clockwise and counterclockwise and it was calculated how each individual s latency time changed

2 /sec 20 3 sec at 4 /sec 24 3 sec and at 8°/sec 25 4 sec (Fig 3)

PATIENTS WITH MENIÈRE S DISEASE

Latency times

Acceleration to the so called healthy ear at 1°/sec gave a latency time of 10 7 sec at 2 /sec 4 4 sec at 4 /sec 2 4 sec and at 8°/sec 2 5 sec (Table I) Here the latency time is decreased by 8 2 sec when the accelera tion strength is increased from 1 /sec to 8°/sec On the other hand if acceleration was directed to the affected ear the following la tency times were obtained at 1°/sec 12 9 sec 2 /sec 8 0 sec 4 /sec 5 3 sec and 8°/sec 1 8 sec i e a decrease of 11 1 sec on increasing acceleration from 1 /sec to 8 /sec (Fig 1)

Since the above values for acceleration to the affected ear partly deviated from the values for acceleration to the healthy ear and also in part differed from the values obtained for the control material calculations were made to ascertain whether any of the differences were statistically significant It was found that the

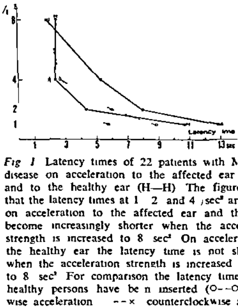


Fig 1 Latency times of 22 patients with Meniere s disease on acceleration to the affected ear (A—A) and to the healthy ear (H—H) The figure shows that the latency times at 1 2 and 4 /sec² are longer on acceleration to the affected ear and that they become increasingly shorter when the acceleration strength is increased to 8 sec² On acceleration to the healthy ear the latency time is not shortened when the acceleration strength is increased from 4 to 8 sec² For comparison the latency times of 22 healthy persons have be n inserted (O--O clockwise acceleration --× counterclockwise accelera tion) and it is evident that these decrease only com paratively slightly on increasing acceleration from 4 to 8 sec²

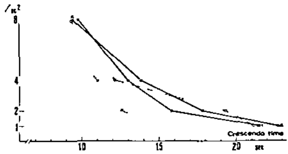


Fig 2 Crescendo times fo 22 patients with Menière s disease The figure shows that the crescendo times are approximately the same on acceleration to the affected and to the healthy ear and that the crescendo times become increasingly shorter when stimulation intensity is increased For comparison the crescendo times of 22 healthy persons have been inserted in the figure For key to the symbols see Fig 1

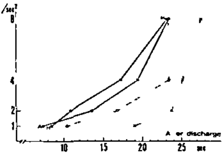


Fig 3 After-discharges in 22 patients with Meniere s disease The figure shows that the after-discharges are about the same on accele ation to the affected and to the healthy ear and that throughout the entire acceleration range they become increasingly longer when the stimulation intensity is increased For com parison the after discharges for 22 healthy persons have been inserted and it is evident that these are longer and that relatively there is only a slight in crease on increasing acceleration from 4 to 8 /sec² For key to the symbols se Fig 1

differences between the latency times on ac celeration to the affected and the healthy ear respectively were not statistically significant If on the other hand the values obtained on acceleration to the affected ear were compared with those for the control material it was found that accelerations at 1 /sec² and 4 /sec showed the duration of the latency times to be significantly prolonged at the 5 level compared with the control material On increas ing the acceleration strength from 1 to 8 /sec to the affected ear latency time was shortened more than in the control material and the dif

Table II *Crescendo times (sec) on stimulation at 1 2 4 and 8 /sec*

Case no	1 /sec²	2 /sec²	4 /sec²	8 /sec²	Case no	1 /sec²	2 /sec²	4 /sec²	8 /sec²
					Normals				
	Clockwise				*Counterclockwise*				
1	9	10	6	11	1	11	12	2	4
2	40	27	20	14	2	19	11	16	8
3	40	12	11	6	3	8	10	8	9
4	25	19	20	10	4	16	13	10	10
5	33	21	11	9	5	14	8	7	7
6	23	20	7	10	6	17	7	10	8
7	21	10	10	6	7	18	16	14	10
8	16	19	25	13	8	16	18	18	5
9	15	9	10	9	9	9	9	5	9
10	19	18	8	6	10	4	7	12	12
11	11	13	14	14	11	15	7	14	12
12	14	15	13	8	12	35	16	16	10
13	23	35	15	16	13	10	8	7	4
14	11	34	14	11	14	24	12	15	6
15	7	19	7	8	15	16	15	7	5
16	34	8	4	8	16	15	12	2	8
17	[illegible]	9	6	5	17	12	21	12	3
18	17	24	8	3	18	10	7	11	5
19	24	22	10	6	19	18	21	12	8
20	14	20	16	8	20	22	14	13	5
21	27	40	21	16	21	4	19	17	11
22	28	21	11	10	22	10	14	13	9
$\bar{x}$	21.1	19.3	12.1	9.4	$\bar{x}$	14.7	12.6	11.0	7.6
S D	9.6	8.8	5.5	3.5	S D	6.9	4.6	4.6	2.7
C	0.46	0.46	0.46	0.37	*C*	0.47	0.37	0.42	0.36
					MB Meniere				
	Acc to the affected ear				*Acc to the healthy ear*				
1	19	9	13	10	1	3	15	10	3
2	40	31	26	16	2	13	14	12	14
3	31	[illegible]	13	12	3	14	11	23	12
4	14	24	14	3	4	29	14	16	9
5	40	14	9	8	5	17	12	15	8
6	13	19	25	15	6	9	4	6	9
7	12	10	15	7	7	17	13	8	6
8	37	18	11	9	8	20	6	10	6
9	10	14	6	6	9	40	20	8	18
10	27	9	8	12	10	27	13	7	11
11	14	26	24	13	11	13	24	19	9
12	11	16	15	9	12	20	12	11	5
13	28	7	9	7	13	24	16	4	3
14	24	14	15	7	14	7	17	12	9
15	10	26	14	13	15	40	11	19	13
16	12	13	10	16	16	26	35	16	13
17	27	15	14	7	17	9	10	19	12
18	40	35	9	7	18	40	15	18	11
19	21	10	10	7	19	6	19	10	8
20	13	9	6	5	20	31	26	17	12
21	17	11	[illegible]	10	21	26	24	8	15
22	40	40	30	7	22	40	17	18	9
$\bar{x}$	22.7	17.8	13.8	9.4	$\bar{x}$	21.4	15.8	13.0	9.8
S D	11.2	9.1	6.7	3.6	S D	11.8	6.9	5.2	3.8
C	0.49	0.51	0.49	0.38	*C*	0.55	0.44	0.40	0.39

Table III *Afterdischarge (sec) on stimulation at 1 2 4 and 8 /sec²*

Case no	1 /sec²	2 /sec²	4 /sec²	8 /sec²	Case no	1 /sec²	2 /sec²	4 /sec²	8 /sec²
					Normals				
	Clockwise				*Counterclockwise*				
1	7	15	15	16	1	9	21	16	24
2	0	21	23	25	2	14	21	23	32
3	8	8	15	18	3	16	19	21	22
4	12	25	25	28	4	31	29	34	37
5	9	13	22	18	5	18	30	10	33
6	7	17	24	25	6	12	13	10	24
7	3	11	33	26	7	13	12	20	31
8	5	15	16	9	8	15	24	17	21
9	13	27	38	29	9	43	39	32	43
10	11	11	12	12	10	23	22	19	15
11	22	15	21	30	11	16	21	28	25
12	3	12	10	15	12	3	13	21	14
13	3	15	16	19	13	31	36	36	36
14	14	27	29	28	14	15	36	29	34
15	8	19	34	20	15	5	16	35	15
16	18	24	33	32	16	51	43	38	40
17	8	12	21	26	17	12	13	20	23
18	19	26	34	32	18	26	22	39	28
19	22	27	24	26	19	11	12	22	23
20	12	9	18	18	20	10	23	15	20
21	17	7	25	29	21	27	34	38	34
22	8	13	27	29	22	16	25	30	30
$\bar{X}$	10 4	16 8	23 4	23 2	$\bar{X}$	19 0	23 8	25 1	27 5
S D	6 2	6 6	7 8	6 7	S D	11 8	9 3	9 2	8 2
C	0 60	0 39	0 33	0 29	*C*	0 62	0.39	0 37	0 30
					MB Menière				
Acc to the affected ear					*Acc to the healthy ear*				
1	10	23	23	32	1	21	19	27	27
2	0	14	8	15	2	3	6	15	16
3	24	36	36	33	3	37	22	40	25
4	2	10	14	14	4	0	7	9	14
5	0	7	6	7	5	0	3	6	14
6	3	7	17	23	6	9	0	11	23
7	23	13	21	28	7	7	8	19	22
8	3	10	12	18	8	12	13	11	17
9	—	10	28	26	9	0	0	2	12
10	12	8	27	32	10	7	20	24	34
11	0	10	20	19	11	8	8	19	27
12	6	15	20	29	12	11	22	23	30
13	11	13	19	17	13	17	20	26	27
14	7	32	30	35	14	3	13	18	35
15	8	14	26	32	15	0	4	14	27
16	13	17	35	29	16	6	25	15	28
17	5	14	26	36	17	10	23	35	39
18	0	7	9	20	18	0	0	7	13
19	1	7	14	17	19	2	6	12	16
20	20	21	23	21	20	11	15	23	21
21	7	11	14	21	21	14	5	14	21
22	0	0	0	9	22	—	1	10	22
$\bar{X}$	7 4	13 6	19 5	23 3	$\bar{X}$	8 5	10 9	17 3	23 2
S D	7 6	8 3	9 3	8 4	S D	8 9	8 5	9 4	7 5
C	1 02	0 61	0 48	0 36	*C*	1 05	0 78	0 54	0 32

ference was statistically significant at the 5% level At this assessment 2 patients were excluded whose mode of reaction was abnormal They had namely longer latency times at 8°/sec than at 1°/sec^2

Crescendo times

If the patients were accelerated to the healthy ear then the crescendo time at 1°/sec^2 was 21 4 sec at 2°/sec 15 8 sec at 4°/sec^2 13 0 sec and at 8°/sec^2 9 8 sec (Table II) If on the contrary acceleration was directed to the affected ear the following values were obtained 1°/sec^2 22 7 sec, 2°/sec^2 17 8 sec 4°/sec 13 8 sec, and at 8°/sec 9 4 sec

Here about the same values were obtained when the accelerations were directed to the affected *and to the healthy ear and the values* were also about the same as those for the control material (Fig 2)

After discharges

The values for the after discharges are listed in Table III On acceleration to the healthy ear the mean values were at 1°/sec 8 5 sec at 2°/sec 10 9 sec at 4°/sec^2 17 3 sec, and 8 /sec 23 2 sec By increasing the accele [illegible] from 1 /sec to 8°/sec the after discharge was prolonged by 14 7 sec The different accelerations to the affected ear gave at 1°/sec an after-discharge of 7 4 sec at 2°/sec 13 6 sec at 4°/sec 19 5 sec and at 8°/sec 23 3 sec Increase in acceleration from 1°/sec to 8 /sec gave in these cases an average increase in the after discharge of 15 9 sec

The calculations made showed that there was no significant difference between the values for the accelerations to the affected and to the healthy ear respectively On the other hand there were quite large differences if comparisons were made with the control material The after discharges on acceleration at 1°/sec^2 to the healthy ear were shorter than in the control material and the differences were statistically significant at *the 5 level* At 2 /sec^2 the difference was statistically significant at the *0 1% level* and at 4°/sec the difference was significant at the 1% level At 8°/sec there was no significant difference When comparing the results of acceleration to the affected ear with the values for the control material it was found that at 1° and 2°/sec^2 the after discharge was shorter than that of the control material and this difference was statistically significant at the 1% level At 4°/sec the difference was statistically significant at the 5% level whereas at 8°/sec there was no difference at all When comparing the prolongation of the after discharges on increasing the acceleration from 1 to 8°/sec in patients with Meniere s disease with that of the control material it was found that this prolongation was greater in the patients with Meniere s disease and the differ*ence was statistically significant at the 5%* level on acceleration to both the healthy and the affected ear

DISCUSSION

If the results in the control material are compared for clockwise and counterclockwise acceleration certain characteristic differences will be found The subjects have throughout longer latency times and longer crescendo times for clockwise accelertion Moreover they have shorter after discharges on acceleration in the same direction If on the other hand we compare how these different components are changed when acceleration is increased from 1° to 8°/sec we find that this change is greater with regard to latency time for clockwise acceleration and with regard to crescendo time also for clockwise acceleration Besides we find that the corresponding change in the after-discharge is also greater than for counterclockwise acceleration This indicates that in normal persons it is more difficult to elicit nystagmus to the right or in other words the right vestibulo-ocular reflex arc offers throughout greater resistance in respect of all the parameters studied in this investigation On the *other hand the differences in the times at 1°* and 8°/sec^2 show that the right vestibulo-ocular

reflex arc has a tendency to react as vigorously to more intense stimuli as the left reflex arc One can ask oneself whether this difference in the results between clockwise and counterclockwise acceleration is due to the fact that the investigation was conducted throughout in such a way that each acceleration was begun by a clockwise acceleration However earlier investigations have shown that this tendency for a nystagmus preponderance to the left is independent of the direction of the initial acceleration (Fluur et al 1967)

Patients with Meniere's disease have shown results which in many respects deviate significantly from those obtained when investigating a normal material The latency times for the material consisting of patients with Meniere's disease were remarkably long on acceleration at 1 /sec both to the affected and to the healthy ear If we then look at the results for acceleration at 2 4 and 8 /sec we find that latency time on acceleration to the healthy ear agrees almost entirely with the values for normal persons The d crease in latency time when increasing acceleration from 1°/sec to 8 /sec is owing to the long latency time at 1°/sec greater than in the normal subjects but is not significant The latency time on acceleration to the healthy ear thus deviates from that of the control material only for very weak stimuli nor is this difference statistically significant however although there is a distinct numerical difference If on the other hand the results are studied on acceleration to the affected ear the values show considerably weaker reactions at 1 2 and 4 /sec whereas at 8 /sec the results entirely agree with those of normal subjects This indicates that the latency time with rising acceleration strength to the affected ear exhibits a clear sign of recruitment

If the crescendo times are compared on acceleration to the affected and the healthy ear respectively these show a high degree of agreement The values are also found to agree well with those of the normal material This may appear rather surprising since the conception crescendo time according to the definition includes latency time which as is evident from the above shows appreciable differences It has not been possible to explain the cause of this The good agreement between the result for the material consisting of patients with Meniere's disease and those of the control material shows that there is no tendency to recruitment in respect of crescendo time

Finally if after-discharges are studied in patients with Meniere's disease it will be found that several characteristic differences from the normal material exist On acceleration to both the healthy and the affected ear the subjects have weaker reactions than in the normal material on accelerations at 1° 2° and 4 /sec On acceleration at 8°/sec however the patients with Meniere's disease display to a great extent the same values as the healthy persons Consequently the after-discharges show clear signs of recruitment at acceleration both to the affected and to the healthy ear

A rotatory test means an increase of activity in one labyrinth and a decrease in the other The investigation shows that the nystagmus which is a result of the integrated reaction from both labyrinths is influenced both on rotation to the healthy and to the affected ear Besides the occurrence of recruitment in patients with Meniere's disease the investigation has shown that the after discharge more definitely emphasizes the occurrence of recruitment than does latency time On the other hand it does not show the same distinct lateral differences as are evident in latency time

ZUSAMMENFASSUNG

Patienten mit einseitiger Menière's Krankheit wurden mit Hinsicht auf vestibulares Recruitment untersucht Sie wurden mit 1 2 4 und 8 /sec² nach rechts und links acceleriert Nach jeder Beschleunigung ging man zu einer konstanten Geschwindigkeit über Zwischen jeder neuen Stimulierung lag ein Intervall von etwa 6 min Man studierte Latenz und Einschwingungszeiten sowie Nachentladungen Die Resultate wurden mit denen verglichen die man von einem Kontroll material von gesunden Individuen erhalten hatte

Bei Beschleunigung zum kranken Ohr hin waren die Latenzzeiten des Menière kranken Individuums

langer als bei Beschleunigungen in Richtung zum gesunden Ohr Der Unterschied war jedoch nicht statistisch signifikant. Verglichen mit den Latenzzeiten des Kontrollmaterial waren sie dagegen statistisch signifikant langer bei Beschleunigungen von 1 und 4 /sec Als man die Beschleunigungsstärke zum kranken Ohr hin von 1 -8 /sec erhohte verminderte sich die Latenzzeit signifikant mehr als er der Fall bei Beschleunigungserhohung im Kontrollmaterial war

Die Einschwingungszeiten waren gleich bei Beschleunigung sowohl zum kranken als auch zum gesunden Ohr hin und waren etwas langer als die des Kontrollmateriales

Die Nachentladungen waren gleich lang bei Beschleunigungen zum kranken als auch zum gesunden Ohr hin Bei 1 2 und 4 /sek waren sie statistisch signifikant kurzer als die Nachentladungen des Kontrollmaterials Bei Erhohung der Beschleunigung von 1 bis 8 /sek² verlängerten sich die Nachentladungen statistisch signifikant mehr als es bei denen des Kontrollmaterials geschah

Die Untersuchungen haben somit gezeigt dass Patienten mit einseitiger Menieres Krankheit ein positives vestibulares Recruitment zeigen sowohl was Latenzzeiten als auch Nachentladungen betrifft

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LABYRINTHINE AND EXTRA LABYRINTHINE EFFECTS ON OCULAR COUNTER ROLLING

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Abstract O ular torsion was measured in alert monkeys There was about 5 –7 of compensatory ocular counter rolling (CR) when the head and body were tilted 45 to the right or to the l ft CR was th same whether the head or head and body were tilted and was not much different in the presence or absence of vision One monkey was tested after serial d struction of the labyrinths Counterclockwise CR was reduced after left labyrinthectomy and clockwise CR after right labyrinth ctomy That is each labyrinth appeared to exert the strongest effect on CR to the opposite side After bilateral labyrinthectomy small but definite amounts of CR were induced by head or by head and body tilt This has been designat d as residual CR In the intact animal CR is mainly induced by the labyrinths In the absence of the labyrinths however other types of sensory activity can influence the steady angle of torsion of the eyes

Ocular counter rolling (CR) is induced when the head is tilted from side to side in the coronal plane The eyes tort or roll to oppose the head movement in an attempt to maintain the absolute position of the retina in space Sensory activity arising in cervical dorsal roots can induce nystagmus or changes in eye position (Barany 1906 Philipszoon 1962 Biemond & de Jong 1969 Takemori & Suzuki 1969) but it is not clear whether extralabyrinthine sensation can evoke CR In the past large angles of CR were sometimes attributed to summation of activity arising in labyrinthine and cervical sensory receptors Recently Nelson (1971) found no effect of cervical sensation on this reflex The present study was undertaken to obtain data on CR in normal monkeys and to study the relative importance of vestibular visual and neck receptors in inducing it

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METHODS

Alert rhesus monkeys (Macaca mulatta) were used in these experiments Stainless steel bolts were screwed into the skull under anesthesia and a cross was tattooed over the center of the cornea Animals were tested with their heads bolted to a primate chair A cross on a grid attached to the head holder provided a fixed spatial reference just above the eyes It lay in the coronal plane which included the front of the cornea The bottom of the grid is just visible in Fig 1 Amphetamine sulfate (0 5 mg/kg) was given to maintain alertness The pupils were constricted with 2 % Pilocarpine to increase the area of the iris so that the black cross could be seen on a lighter background

Eye movements were recorded with a 35 mm single lens reflex camera fixed to the primate chair The film plane was parallel to the coronal plane Pictures were taken with an eccentric flash at irregular intervals of 5–30 sec The linear distance and angular difference between the reference cross and one arm of the

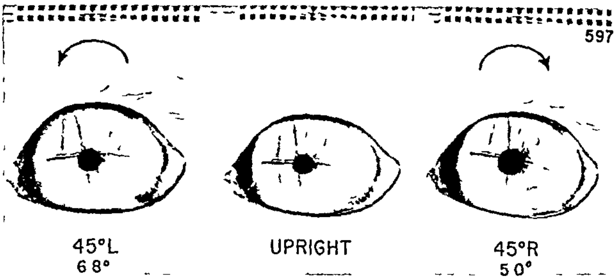


Fig 1 Photographs showing compensatory counter rolling in Monkey 597 against head and body tilts of 45 R and 45 L.

corneal cross were determined from negatives on a microscope with a rotating stage and a projection screen The same arm of the cross was used for measurement in each animal The standard deviation of measurement of the angle between the corneal and reference crosses was about 0 5

Because the corneal cross could move out of the film plane when the eye was in different ...ital positions the apparent angle of rotation ...uld change without there having been actual torsion of the eyes This is shown in the three dimensional graph of Fig 2 *A* The length of the horizontal black lines represents the angular difference between the corneal and reference crosses The shorter the line the more the corneal cross appeared rotated in a counter clockwise direction and vice versa In this animal when the left eye was abducted or de pressed the cross appeared to have more counterclockwise rotation than when adducted or elevated

A linear regression analysis for two independent variables *horizontal and vertical eye position* was used to correct for effects of eye position on the apparent angle of the corneal cross The angular difference between the reference and corneal crosses was the dependent variable After correction there was no relation ship between the position of the eyes and the angle of the corneal cross (Fig 2 *B*) An *F* test was positive showing that correction for horizontal and vertical eye position had signific antly altered the data (> 0 005)

Regression coefficients determined with the animals upright were used to predict the angle of the corneal cross in each eye position The actual value was then compared to the pre dicted value to see if there had been additional ocular torsion related to head position About 30–35 samples were used for determining the regression coefficients and for measuring CR in each position.

Monkeys were tested under the following conditions (1) *Head and body tilt* (*H* & *B*) The entire animal was tilted 45 to the right or to the left (2) *Head tilt* (*H*) With the body vertical the head was tilted 45° right or left To accomplish this the primate chair was tilted 45 in one direction and the body was counter tilted in the other This maintained the rela tionship between the eye cross the head holder and the camera (3) *Body tilt* The body alone was tilted approximately 90 (4) *Body rota*

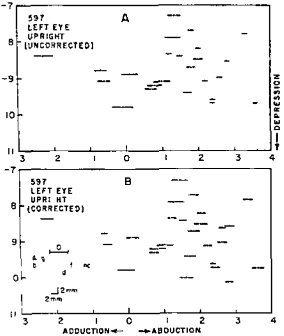


Fig 2 Three dimensional graphs showing the angular difference between the reference cross and corneal cross with the left eye in different initial positions before (*A*) and after (*B*) correction for horizontal and vertical eye position The abscissae and ordinates correspond to horizontal and vertical eye position in the coronal plane measured on the microscope stage The length of each horizontal bar is the magnitude of the angular difference between the reference and corneal cross A fixed amount has been added to all of the values to make them positive Eye positions in the direction of adduction abduction and depression are marked After the regression analysis (*B*) there was no consistent relationship between the angle of the corneal cross and eye position

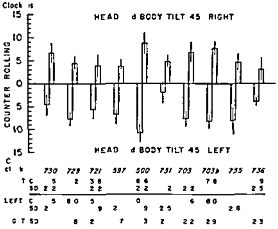


Fig 3 Graph showing the mean CR opposing head and body tilt of 45 to the right (above the abscissa) and left (below the abscissa) The vertical line through the top of each bar shows one standard deviation above and one below the mean Numerical values are shown in the table below

tion The body was rotated approximately 90 to the right or left (5) *Upside down* Animals were inverted (6) *Light and darkness* Most testing was done in darkness interrupted only by the strobe flashes used to photograph the eyes In several experiments the room was continuously lighted

Unilateral and bilateral labyrinthectomies were performed on several animals Each part of the labyrinth was separately visualized and destroyed under an operating microscope After labyrinthectomy animals were tested after spontaneous and positional nystagmus in darkness had subsided The completeness of the bilateral labyrinthine destructions was verified physiologically There was no response to rotation in darkness and positional alcohol nystagmus (PAN) was absent Cervical dorsal root section (CDRS) was also performed under the operating microscope Posterior roots C2 C3 C4 and usually C5 were individually visualized and cut at their point of entrance into the cervical spinal cord

At the conclusion of the experiments when monkeys were killed the diameter of the eye balls was measured From this it was possible to relate linear distance in the coronal plane to degrees of horizontal or vertical ocular deviation

RESULTS

When monkeys were tilted in darkness the eyes opposed the head and body tilt by rolling or torting about 5 –7 in the opposite direction Examples of CR against 45 of head and body tilt in Monkey 597 are shown in Fig 1 In 9 animals tested on 10 occasions (Fig 3) mean CR was 5 4 against head and body tilt to the right and 6 5 against head and body tilt to the left The largest angles of CR were 8 6 and 10 8 in Monkey 500 CR was measured in Monkey 703 twice and was similar on both occasions (Fig 3 703*a* & *b*) In Mon

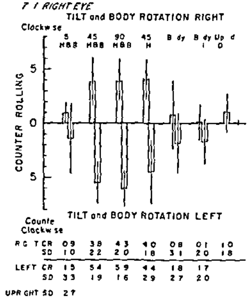


Fig 4 CR induced by head and body tilts (H & B) of 5 45 and 90 and by head tilt (H) of 45 The angle of ocular torsion was also measured with the body rotated on the neck (Body rot) with the body tilted 90 but with the head upright (Body tilt) and with the animal upside down The dotted line at the right shows two standard deviations of angular eye position with the animal upright

key 731 counterclockwise CR against head and body tilt to the left was small (1 9 ±2 5°) This animal did not have positional nystagmus in darkness and its caloric responses were intact and appeared normal Standard deviations of angular eye position were similar whether animals were tilted or were upright The mean standard deviation in the right lateral position was 1 9 in the left lateral position was 2 0 and in the upright positions was 2 1 Some of this variation was probably due to spontaneous torsional movements (Miller 1962 Nelson 1971)

Ocular torsion was also measured during smaller and larger angles of tilt (Fig 4) Although there was a tendency for CR to occur in the expected direction after 5 of head and body tilt differences in rotatory eye position in the upright and tilted positions were not significant ($p < 0\ 05$) CR induced by head and body tilts of 45° and 90 was not different showing that the mechanism for its production was almost saturated by 45 of tilt

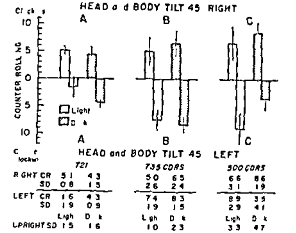


Fig 5 CR induced by head and body tilt in light (clear bars) and in darkness (cross hatched bars) in Monkeys 721 (*A*) 735 (*B*) and 500 (*C*) 735 and 500 had had section of the cervical dorsal roots prior to testing but the labyrinths were intact in all 3 animals

Effects of vision on CR were tested in continuous room illumination and in darkness interrupted only by the strobe flashes (Fig 5) There was no consistent difference in CR in light and in darkness CR was somewhat larger in darkness than in light in Monkey 735 but these differences were not significant ($p < 0\ 05$)

Effects of cervical sensation on CR

Neck sensation had little apparent effect on CR in the intact animal CR and standard de

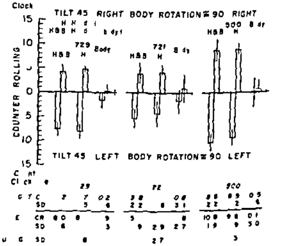


Fig 6 CR in Monkey 729 721 and 500 induced by 45 of head and body tilt (H & B) and 45 of head tilt (H) Angular eye position after 90 of body rotation is also shown

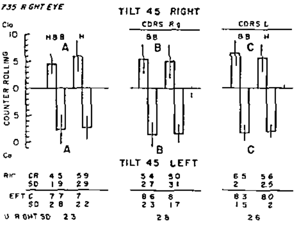


Fig 7 CR in Monkey 735 induced by 45 of head and body (H & B) or head tilt (H) Data was taken (*A*) before operation (*B*) after unilateral cervical dorsal root section (CDRS Right) and (C) after bilateral CDRS (CDRS Left)

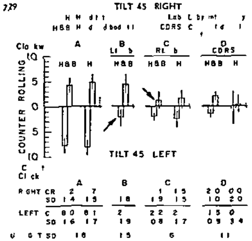


Fig 8 CR in Monkey 729 (*A*) before operation (*B*) after left labyrinthectomy (Lt. lab) (*C*) after right or bilateral labyrinthectomy (Rt lab) and (*D*) after bilateral cervical dorsal root section (CDRS)

viations of CR were the same whether the head and body (H B) or only the head (H) were tilted (Figs 4 6) Neither body rotation nor body tilt alone induced significant amounts of CR (Figs 4 6) CR was also measured be fore and after unilateral and bilateral CDRS (Fig 7) The amount of CR induced by head (H) or by head and body (H & B) tilt was un affected by CDRS on one or on both sides

Effects of labyrinthectomy on counter rolling

CR in Monkey 729 was studied before and after serial destruction of the labyrinths Be fore operation there was about 4 5 of clock wise CR against tilts to the right and about 8 of counterclockwise CR against tilts to the left (Fig 8 *A*) The left labyrinth was destroyed first Three weeks after operation when ny stagmus to the right in darkness had subsided counterclockwise CR with the left side down was reduced from 8 to 2 (Fig 8 *B* upward arrow) Clockwise CR induced with the right or intact side down was preserved after left labyrinthectomy One week after the right labyrinth was destroyed clockwise CR to the right was reduced from about 4 5 to about 1 5 (Fig 8 *C* downward arrow)

Although CR was reduced after bilateral labyrinthectomy in 729 it was not completely abolished That is the eyes tended to be deviated in the compensatory direction when either the head or head and body were tilted (Fig 8 *C*) Differences in mean angular eye position in the upright and tilted positions were not significant However there were significant differences in the angle of torsion of the eyes in the right and left tilt positions ($p > 0\,001$) They were 3 9 when the head was tilted and 3 4 when the head and body were tilted CR remaining after bilateral labyrinthectomy has been designated as residual CR Residual CR after bilateral labyrinthectomy was also present in Monkey 707 (Fig 9 Bilat lab) Differences in mean angular eye position during head and body tilt and during head tilt (5 9 & 6 6) were also significant ($p > 0\,001$)

The cervical dorsal roots were sectioned bilaterally in Monkeys 729 and 707 to deter mine whether or not neck sensation had con tributed to production of residual CR In 729 the difference in mean eye position was similar when the head and body were tilted together before and after CDRS (Fig 8 *C D H & B*) However no CR was recorded after CDRS when only the head was tilted (Fig 8 *D H*) Similarly CR was reduced in Monkey 707 after bilateral labyrinthectomy and CDRS when only

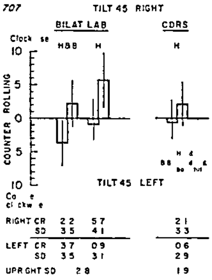


Fig 9 CR in Monkey 707 after bilateral labyrinthectomy (Bilat lab) and cervical dorsal root section (CDRS)

the head was tilted (Fig 9 CDRS) Thus neck sensation could influence production of CR under certain circumstances but was not essential for its presence

Horizontal and vertical eye position

Since the technique used for measuring CR did not require visual fixation eye positions in the and vertical planes were recorded concomitantly with angular eye position There was no remarkable changes in horizontal or vertical eye position during lateral tilt although there was some tendency for the eyes to be on the side of the midline in the direction of tilt particularly when the head was bent on the neck When animals were inverted there was a significant downward deviation of the eyes in three monkeys ($p > 0\ 025$) In one of the animals (707) both labyrinths had been destroyed before testing This indicates that while the labyrinths may have contributed to the ocular depression when upside down they were not solely responsible for it

DISCUSSION

In humans about 5 –7 of CR is induced by head and body tilts of from 45 to 90 (Woellner & Graybiel 1959 Miller 1962 Miller & Graybiel 1965 Hannen et al 1966 Nelson 1971) Similar amounts of CR were induced in alert monkeys Data in humans were taken while subjects were fixating a target and in monkeys with and without visual fixation The similarity in results suggests that visual fixation or lack of it does not significantly alter the angle of steady compensatory ocular torsion induced by lateral head and body tilts In monkey as in man CR against head and body tilt is small and does not adequately compensate for the extent of retinal displacement caused by lateral head positions The imperfect nature of the compensatory mechanism is emphasized by the finding that the amount of CR against 5 of head and body tilt was barely recognizable and that the mechanism was essentially saturated by head and body tilts of 45°

CR in the intact monkey was the same whether the head was tilted on the neck or the head and body were tilted together This is consistent with findings of Nelson (1971) In intact individuals CR appears to arise primarily from activity of labyrinthine sensory receptors and cervical visual and somato-sensory afferents would appear to contribute little to production of it Miller (1962) has inferred from the anatomy of the labyrinth that CR is induced mainly by activity originating in the utricle Under most circumstances therefore CR can probably serve as one index of the functional integrity of the utricle or of utriculo-ocular pathways

The findings are also in agreement with studies that show that clockwise CR is mainly induced by the right labyrinth when right side is down and vice versa (Versteegh 1927 Kusakari 1969 Suzuki et al 1969 Nelson 1971) In most humans Nelson (1971) found a persistent asymmetry in CR after unilateral vestibular nerve damage or nerve section CR in the direction opposite the damaged side was reduced when that side was down In cats after unilateral labyrinthectomy CR was also initially absent to the contralateral side but later

adapted and was reported to be symmetrical though reduced (Kusakari 1969)

A new finding of this study is that small but definite amounts of CR were present in the absence of the labyrinths The origin of this residual CR is not entirely clear but three possible sources are from activity in neck receptors from extra cervical somato-sensory afferents responsive to gravity and from the visual system Although there is some evidence to suggest a possible role for each of these sensations it is too fragmentary to permit any conclusion Small angles of CR were also present in some labyrinthine defective (L–D) humans when tilted with head neck and body immobilized (Miller & Graybiel 1963) It is unlikely that the residual CR in these L–D subjects had originated in neck receptors be cause of the rigid fixation of head and neck It is also unlikely that it arose in the visual system since it increased when G forces were increased by centrifugation (Miller & Graybiel 1965) It is possible that it arose in extra cervical somato-sensory afferents However all labyrinthine function may not have been totally absent in these persons and this is a subject for further study

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ZUSAMMENFASSUNG

Bei wachen Affen wurde die okulare Torsion ge messen 5–7 kompensatorische Augengegenrol lung bei 45gradiger Kopf und Korperbeugung nach rechts oder nach links wurde gefunden Die Summe der Au engegenrollun, bei der Kopfbeugun, oder Kopf und Korperbeugung wurde nicht veran dert Die Augengegenrollung war nicht bedeutend von der An oder Abwesenheit des Sehens abhangig Ein Affe wurde nach einseitiger und dann nach beider seitiger Labyrinthzerstorung unt rsucht. Di Augen gegenrollung gegen die Uhrzei rrichtung wurde nach der linkseitigen und in der Uhrzeigerri htung nach der rechtseitigen Labyrinthectomie herabgesetzt d h jedes Labyrinth führt den starkesten Einfluss auf die Gegenrollung in der entgegeng setzten Seite aus Nach der beiderseitigen Labyrinthectomie wurden noch kleine Werte der Gegenrollung bei der Kopfbeugung oder bei der Kopf und Korperbeugung gefunden Wir haben diese Werte als die Restaugengegenrollung bezeichnet Bei einem normalen Versuchstier ist nor malerweise die Augen egenrollung meistens aus dem Labyrinth hervorgerufen Bei der Abwesenheit beider Labyrinthe konnen jedoch die anderen Typen der s nsorischen Aktivitat den standigen Winkel der Au gentorsion beeinflussen

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THE COCHLEAR BLOOD VESSELS IN GUINEA PIGS OF DIFFERENT AGES

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Abstract The vascular pattern of the cochlea was compared in guinea pigs of different ages by a previously presented method of contrast injection The anatomical findings are similar in the newborn and old guinea pigs which is consistent with the very sparse information published on the subject The appearance of the vessel of the basilar membrane also called vas spirale is similar in newborn and old guinea pigs in spite of its large embryonic size Differences between young and old animals consisted of a thicker bone in the bulla and cochlear capsula a more frequent occurrence of otitis media and of pigment surrounding the spiral modiolar artery in the old animals Consequently the newborn and young animals can be recommended for vascular studies

The vascular anatomy of the guinea pig cochlea has been described in detail (Axelsson 1968) Very little is published comparing the cochlear vascular anatomy of young and old guinea pigs Leimgruber (1903) stated that the stria vascularis was similar in newborn and adult animals Covell & Rogers (1957) investigating aged guinea pigs with a diminished or absent Preyer s reflex did not find any changes in the walls of the modiolus vessels although there was an increase in the amount of perivascular connective tissue There was no consistent finding in the stria vascularis which was normal in many animals In some however atrophic changes increased pigmentation and cystic degeneration were observed in the stria vascularis

Degenerative changes in other mammals due to age have been described and particularly in the human cochlea and consist of degeneration of nervous elements afferent as well as efferent centrally as well as peripherally The following vascular changes due to age have been reported Atrophy of the stria vascularis in the lower basal turn and a thickening of the vascular wall in the meatus vessels (Fabinyi 1931) arteriosclerosis of the internal auditory artery (Bozzi 1934) angiosclerotic degeneration of the stria vascularis (Saxen 1952) PAS positive thickening of the capillary walls of the stria vascularis (Jorgensen 1961) diminished blood flow through the acoustic mechanism due to atherosclerosis and capillary angiopathy with interference in the capillary exchange of nutrients and removal of waste products (Rosen et al 1964) slight vascular alterations throughout the cochlea simultaneous with more marked changes in the vessels of the internal auditory meatus the circle of Willis and the peripheral vessels of the cerebrum (Hansen & Reske Nielsen 1965) and atrophy of the stria vascularis in the apical half of the cochlea with changes in the bioelectrical biochemical and energy producing properties of the endolymph (Schuknecht 1964 Schuknecht & Ishii 1966) The aim of the present investigation has been to examine the vascular anatomy of the cochlea in guinea pigs of different ages



Fig 1 Old guinea pig Spiral modiolar artery and the so-called glomeruli (left) i e the coiled proximal parts of the radiating arterioles in the modiolus Note the distribution of pigment around the artery

MATERIAL AND METHODS

70 guinea pigs of different ages were examined grouped as follows (A) 22 newborn animals less than 1 week old weighing less than 125 g (B) 10 young animals 1–8 weeks old weighing 125–250 g (C) 8 adult animals 2 to several months old weighing around 250 g (D) 30 old animals 2–3 years of age weighing more than 1 000 g The animals were picked at random without testing the hearing

The animals were prepared according to the following method described previously in detail (Axelsson 1968) A plastic catheter is introduced in the ascending aorta of the anesthesized guinea pig The animal is bled and subsequently perfused with Ringer s solution and then with a contrast medium 3° Berlin blue solution The subsequent preparation contained the following steps fixation decalcification neutralization washing dehydration storage in glycerin Microdissection of the cochlea was performed in the stereo-microscope and photographic registration of the vascular anatomy was achieved by the aid of the photomicroscope (Leitz Ortoplan) All parts of the cochlea were thoroughly examined in the stereo- as well as in the photo-microscope Phase-contrast microscopy was used in order to make uninjected vessels visible Berlin blue was also attempted to demonstrate the vessels of the embryonic cochlea in pregnant guinea pigs The contrast however did not pass through the placenta to the foetus

RESULTS

Differences between newborn and old animals were apparent when the bulla was opened The cochlea of the old guinea pigs required more decalcification than in the young animals due to that the bulla and bony cochlear capsula are thicker than in the newborn animal In the latter the bulla always contained air and was free of infection whilst in the old animals an

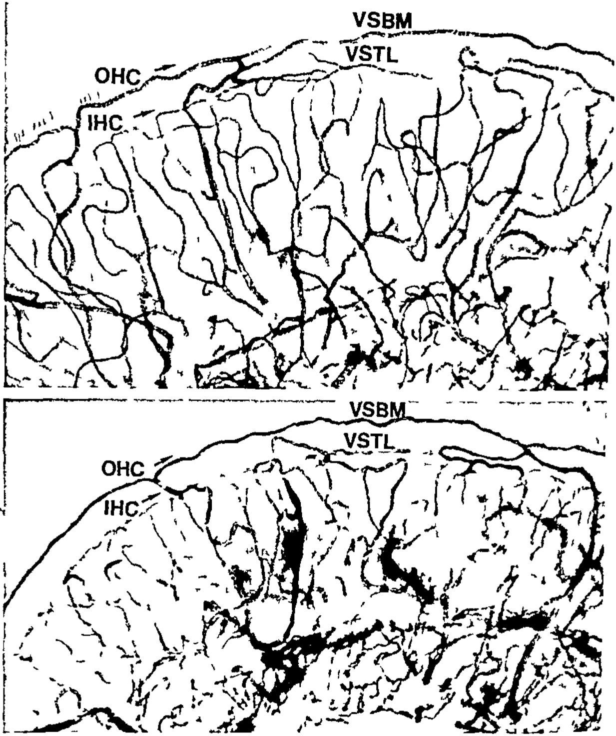


Fig 2. Newborn guinea pig (above) and old (below). Spiral lamina basal turn. *VSBM* vessel of the basilar membrane. *VSTL* vessel of the tympanic lip. *OHC* outer hair cells. *IHC* inner hair cells.

otitis media or a healed middle ear infection was a common finding. In healed otitis media the periosteum was thickened and the vascular pattern changed. Many delicate vessels form an abnormal dense vascular pattern. Even when the whole middle ear contained pus the periosteum vessels usually were well injected. Another common finding which differentiated

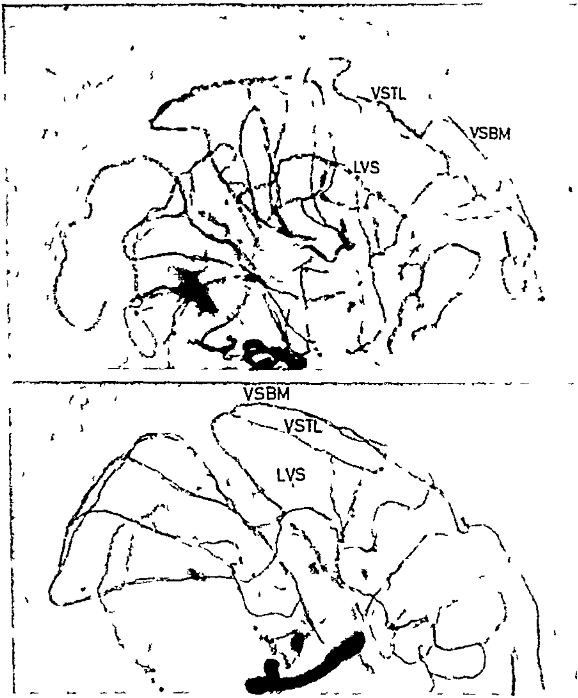


Fig 3 Spiral lamina 4th turn of the newborn (above) and old (below) guinea pig *VSBM* vessel of the basilar membrane situated immediately below the outer hair cells *VSTL* vessel of the tympanic lip *LVS* limbus vessels The apical simplification is similar and all vessels present

old from young guinea pigs is the occurrence of pigment which is patchy distributed around the spiral modiolar artery in the modiolus Ramifications of pigment often more or less surround the artery ending with a small cup or foot (Fig 1)

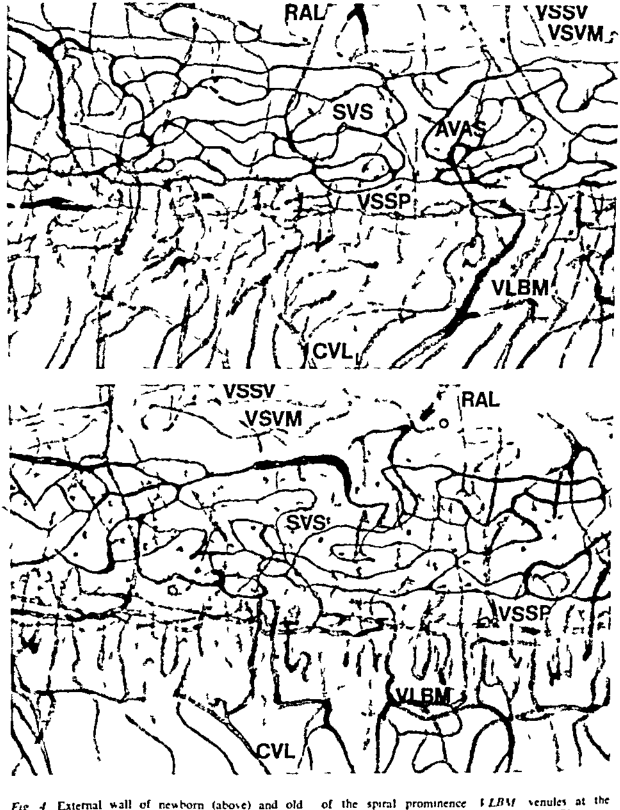


Fig 4 External wall of newborn (above) and old guinea pig (below) Basal turn *RAL* radiating arterioles. *VSSV* vessel of the scala vestibuli *VSVM* vessel at the vestibular membrane *SVS* stria vascularis *AVAS* arteriovenous anastomoses *VSSP* vessel of the spiral prominence *VLBM* venules at the basilar membrane *CVL* collecting venules The same vessels can be identified in the newborn and old animal

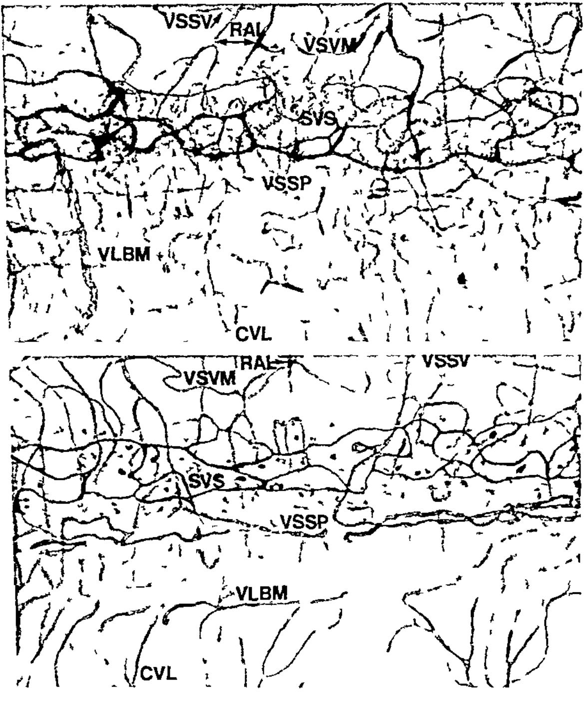


Fig 5 External wall of the newborn (above) and the old guinea pig (below) Third turn *RAL* radiating arterioles *VSSV* vessel of the scala vestibuli *VSVM* vessel at the vestibular membrane *SVS* stria vascularis *VSSP* vessel of the spiral prominence *VLBM* venules at the basilar membrane *CVL* collecting venules The same vessels can be identified in the newborn and old animal Note the decreasing width of the stria vascularis

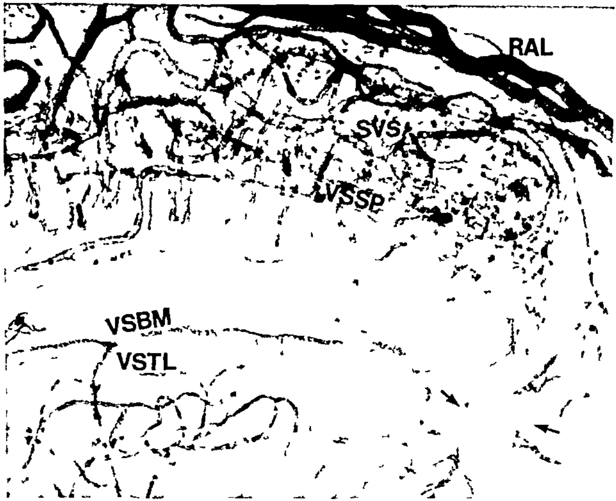


Fig 6 Old guinea pig Extreme basal end *RAL* radiating arterioles turning around the extreme basal end of the spiral lamina and running an apical "retrograde" direction to supply this region *SVS* stria vascularis *VSSP* vessel of the spiral prominence *VSBM* vessel of the basilar membrane *VSTL* vessel of the tympanic lip *SVS* and *VSSP* are drained by collecting venules at the extreme basal end (*arrows*)

All previously described regularly identifiable vessels of the membranous cochlea could be demonstrated in all age groups and in all four cochlear turns (Table I) The apical simplification of the vascular pattern in the spiral lamina and in the external wall as well as the particular vascular pattern at the basal end were similar in all age groups (Figs 2–6) The appearance of the vessel of the basilar membrane did not differ in the newborn and aged cochlea (Figs 2 3) As in the aged cochlea there are in the newborn animal no vascular connections between the stria vascularis and the vessel of the spiral prominence thus indicating that these are separate vascular systems (Figs 4 5) In 8 out of the 70 cochleas examined and in all age groups a vessel could be demonstrated running through the scala tympani from the modiolus to the collecting venules of the external wall (Fig. 7) This vessel is situated near the round window membrane It was not possible to follow its origin in the modiolus bone

Even if all regularly identifiable vessels are demonstrated in the different age groups detailed studies reveal that no two cochleae are similar as demonstrated by the present method Sometimes the arterial sometimes the venous side is better filled by contrast In certain areas

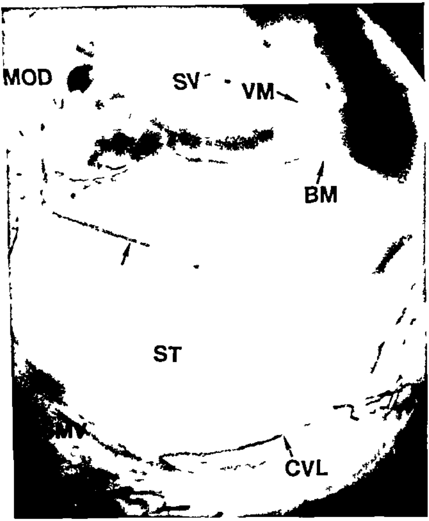


Fig. 7. Basal turn apico-basal section. Newborn guinea pig. A vessel (*arrow*) runs from the modiolus (*MOD*) through the scala tympani (*ST*) to the collecting venules (*CVL*) in the external wall. *SV*: scala vestibuli. *VM*: vestibular membrane. *BM*: basilar membrane. *SMV*: spiral modiolar vein.

of the cochlea the injection can be relatively defect while nearby areas apically and basally are well filled. The majority but not all the capillaries of the stria vascularis are contrast-filled which is disclosed by the phase-contrast microscopy. In other preparations the capillaries may seem dilated by stasis. However, this is seen in all groups. The vascular pattern of the cochlea thus is similar in guinea pigs of all ages.

DISCUSSION

The present investigation was aimed to compare the vascular pattern of the cochlea in guinea pigs of different ages. The most complete contrast filling of the vessels was achieved in the newborn guinea pigs. The reason for more failures among the old animals was in part more frequent ruptures of the vessels in the inner acoustic meatus. The frequent occurrence of middle ear infection, however, did not seem to influence the result of the injections.

As evidence of a normal anatomy, anatomical findings should be demonstrated regularly in different age groups. There is a great lack of anatomical investigations comparing the vascular anatomy of the cochlea in young and old guinea pigs in spite of the frequent use of this animal for experiments. In well contrast-filled cochleae of all age groups the previously described vascular pattern of the cochlea could

Table I *Regularly demonstrable vessels of the guinea pig cochlea*

Modiolus
Spiral modiolar artery
Radiating arterioles
Spiral modiolar vein
Collecting venules
Capillaries of the acoustic nerve
Capillaries of the spiral ganglion
Capillaries of the wall of the modiolus
Spiral lamina and limbus
Radiating arterioles
Collecting venules
The vessel of the basilar membrane
The vessel of the tympanic lip
External wall
Scala vestibuli
Radiating arterioles
Collecting venules
The vessel of the scala vestibuli
The vessel at the vestibular membrane
A capillary net above the vestibular membrane
Scala media
Stria vascularis
The vessel of the spiral prominence
Arterio-venous anastomoses
Scala tympani
Collecting venules
The venules at the basilar membrane

confirmed in the present investigation Even if the principal pattern can be confirmed in every cochlea there are regular individual variations due to small differences not only in the vascular pattern but also in the degree of contrast filling probably due to undefined differences in the technical performance and not to pathological vascular alterations

In several cochleae a vessel could be demonstrated running from the modiolus to the collecting venules of the external wall of the scala tympani This vessel curiously runs through the perilymph of the scala tympani Similar findings have been reported by Wolff (1935) in the human and by Maass (1969) in the guinea pig cochlea It is supposed to be an embryonic remnant from the foetal vascular development of the cochlea The vessel of the basilar membrane also called vas spirale has been described to be of considerable diameter in the embryo (Baginsky 1886 Boettcher 1887, Hilding et al 1967 Kikuchi & Hilding 1967 Bredberg 1968) In the adult guinea pig this vessel is only around 5 microns when measured after injection of contrast medium (Axelsson 1968) Since the cochlea is completely developed some time before birth it is of interest to investigate whether the vessel is of adult calibre in newborn animals The vessel was found to be of the same appearance in the newborn as in the old guinea pig

No changes due to age could be demonstrated in the vessel of the basilar membrane situated close to the organ of Corti nor in the stria vascularis probably of great importance for the secretion and absorption of endolymph Neither could any changes be demonstrated by the present method in the particular and highly vascularized region of the basal end Consequently the findings of the present investigation are in agreement with findings of Leimgruber (1903) and Covell & Rogers (1957) who did not find any pronounced and regularly occurring changes in the vessels of the aged guinea pig cochlea

It is not known if the hearing deteriorates due to age in the guinea pig If there is a presbycusis in the guinea pig the present investigation reveals that it is not due to gross vascular anatomical changes in any part of the cochlea

The consistent occurrence at different ages of the previously defined cochlear vessels is further proof that this vascular pattern constitutes a normal vascular anatomy The vascular pattern of the guinea pig cochlea is thus unchanged from birth up to advanced age The more regularly successful contrast injection in the newborn animals makes them more suitable for vascular studies than the old ones

ZUSAMMENFASSUNG

Die Gefässmuster der Schnecke von Meerschweinchen verschiedener Altersstufen wurden mit Hilfe einer früher beschriebenen Kontrastinjektionsmethode miteinander verglichen Die anatomischen Befunde

bei neugeborenen und alten Meerschweinchen waren einander ähnlich. Dieses Resultat stimmt mit den sparsamen Veröffentlichungen in dieser Frage überein. Das Gefäss der Basilarmembran, auch Vas spirale genannt, ist bei neugeborenen und alten Meerschweinchen einander ähnlich, obwohl es im Embryo sehr weit ist. Der Unterschied zwischen jungen und alten Tieren bestand in einem dickeren Knochen in der Bulla und in der Schneckenkapsel, über öfters vorkommenden Otitis media sowie dem Vorkommen von Pigment rings um die A. spiralis modioli alter Tiere. Deshalb können neugeborene oder junge Tiere für vaskuläre Studien der Cochlea empfohlen werden.

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RESPONSES OF SINGLE NEURONS OF THE VESTIBULAR NUCLEI TO LATERAL TILT AND CALORIC STIMULATION IN THE INTACT AND HEMILABYRINTHECTOMIZED CATS

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Abstract Responses of unitary discharges of the ves tibular nuclei to lateral tilt and caloric stimulation were studied in the unanesthetized *encéphale isolé* preparations of intact and hemilabyrinthectomized cats The responses of firing rate of 93 units to lateral tilt were classified into 5 groups increased type (in crease with lateral tilt to either or both directions) decreased type (decrease with lateral tilt to either or both directions) reversed type I (increase with ipsilateral and decrease with contralateral tilt) re versed type II (decrease with ipsilateral and increase with contralateral tilt) and refractory type (refractory with lateral tilt to both directions) The most domi nant pattern of the responses to lateral tilt was the type (60 2) On the other hand more an half of the vestibular units (61 3) showed changes in frequency to caloric stimulation with cold and warm water Two main types of units were localized in the vestibular nuclei one increased the frequency in response to ipsilateral tilt selectively and another increased the frequency in response to *caloric stimulation with cold water* Contralateral labyrinthectomy decreased the firing rate of spon taneous unitary discharges in the vestibular nuclei and the decreased frequency recovered to the control level in 42 days after the operation However the pattern of responses of the vestibular neurons to lateral tilt was not modified by the contralateral labyrinthectomy

Since the earlier works by Ross (1936) and Adrian (1943) a number of observations on the responses of single cells of the vestibular nuclei to afferent stimulation of labyrinth have been reported Vestibular mechanisms in the central nervous system however have not been well understood by comparison with the information on the central auditory mecha nisms The studies by Matsuoka (1967) on the responses of the vestibular neurons to caloric stimulation in both anesthetized and unanesthetized cats demonstrated that the re versed type of response which consisted of an increase in the firing rate in response to caloric stimulation with cold water and a decrease with warm water, was found in only the latter preparation These findings suggested that an ability of the vestibular neurons to differentiate both stimuli was lost under barbiturate anes thesia Further observations of Matsuoka (1969) on the effects of caloric stimulation of the contralateral middle ear cavity on the ves tibular neurons supported a functional connec tion between the bilateral vestibular nuclei It is well known that the semicircular canals respond to angular acceleration of the head and caloric stimulation while the otolith organs are the sources of responses to gravity and linear acceleration Influences of angular ac celeration of the head on the central ves tibular functions were studied by several in vestigators (Gernandt 1949 1959 Shimazu & Precht 1965 1966 Precht et al 1966) The responses of the vestibular neurons to caloric stimulation were reported by Gernandt (1949) Gernandt & Thulin (1952) Oya (1960) and Weber & Steiner (1965) and those to lateral tilt were also presented by Adrian (1943) and Peterson (1967) However there

are few systematic studies on the responses of the same single cells of the vestibular nuclei to stimulation of the receptor structures in the semicircular canals and otoliths

In the present experiments effects of caloric stimulation which provoked convection currents in the endolymph of the semicircular canals were compared with those of lateral tilt which produced a different pull of gravity upon the receptor structures of otoliths on the activity of the same single neurons in the vestibular nuclei of the intact and contralateral labyrinthectomized cats

METHODS

The experiments were performed on 25 *encephale isole* cats of either sex weighing between 3 0 to 3 5 kg They were previously confirmed to be normal in their voluntary movement

Under ether anesthesia the left tympanic bulla was exposed by incision of the neck After trepanation of the middle part of the tympanic bulla about 5 mm in diameter the mucous membrane of the middle ear cavity was removed and tympanic membrane was incised A metal tube of 3 mm in diameter was inserted into the hole of the bulla and was fixed with dental cement Five ml of water at the temperature of 30 C (cold stimulation) and 44 C (warm stimulation) were infused through the metal tube allowing irrigation through the tympanic bulla and middle ear cavity to the external meatus

The head of the animal was fixed on a stereotaxic instrument which was devised by the Brain Research Institute Niigata University School of Medicine The animal was then immobilized by transecting the spinal cord at level of C_1 or C and was maintained on artificial respiration The recording was started at least 4 hours after the termination of the surgical procedures and ether inhalation The stereotaxic instrument and the animal were mounted on a movable goniometer allowing up to 30 inclination in a lateral direction

The recording microelectrode was a glass insulated gold wire 15 to 20 μ in diameter and 0 75 to 1 0 MΩ in electrical resistance The electrode was inserted into the left vestibular nuclei mainly into the Deiters nucleus using a micromanipulator The sites of recording were confirmed histologically after the termination of the experiments

The spontaneous firing rate of each unit was recorded in the horizontal position for 2 min or more and the instrument was tilted to the left i e with the recording side down at a speed of 2 /sec The firing rate was observed in a tilt of 30 from the horizontal level for 2 min and then the instrument was restored to the horizontal position When the firing rate recovered to the previous level usually a few minutes later the instrument was tilted 30° to the right i e with the recording side up at the same speed and held in that position for 2 min Thereafter caloric stimulation with cold and warm water was applied to the same single neuron in the horizontal level following to the method described previously (Matsuoka 1967)

The technique of unilateral labyrinthectomy was as follows The cat was anesthetized with an intraperitoneal injection of 30 mg/kg of pentobarbital sodium The right tympanic bulla was aseptically exposed by a ventral approach and wall of the bulla was resected Under direct visual inspection the round window of the cochlea was removed and the otoliths and the ampullae of semicircular canals were completely destroyed A mixed solution of penicillin and dihydrostreptomycin was given locally and systematically for 3 days after the operation At 7 14 21 and 42 days after the hemilabyrinthectomy the responses of single cells in the contralateral vestibular nuclei to lateral tilt and caloric stimulation were observed in the *encephale isole* preparation

A more detailed description of the experimental procedures may be found in the vious papers (Matsuoka 1967 1969)

RESULTS

Responses of the vestibular neurons to lateral tilt

The spontaneous unitary discharges in the vestibular nuclei consisted of mono- or biphasic spikes of 0.2 to 1.5 mV in amplitude. The majority of the units showed a firing rate of 5 to 30/sec and the mean firing rate of 93 units was 18.4±1.2 (S.E.)/sec.

The responses of the unitary discharges of the vestibular nuclei to lateral tilt were classified into the following 5 groups: 1) the increased type in which lateral tilt to either or both directions produced a significant increase in the firing rate; 2) the decreased type in which lateral tilt to either or both directions produced a significant decrease in the firing rate; 3) the reversed type I in which the firing rate increased with lateral tilt to the left and decreased with tilt to the right; 4) the reversed type II in which the firing rate decreased with lateral tilt to the left and increased with tilt to the opposite direction.

Table I. *Responses of the same single units of the vestibular nuclei to lateral tilt in the intact cat*

Types	In left tilt	In right tilt	No. of units	( )
Increased type	Increase	Increase	7	10 (10.8)
	Increase	No change	2	
	No change	Increase	1	
Decreased type	Decrease	Decrease	50	56 (60.2)
	Decrease	No change	4	
	No change	Decrease	2	
Reversed type-I	Increase	Decrease	4	(4.3)
Reversed type-II	Decrease	Increase	19	(20.4)
Refractory type	No change	No change	4	(4.3)
Total			93	

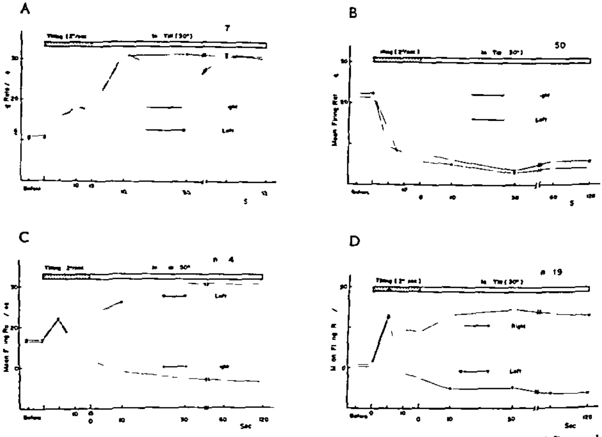


Fig. 1. Responses of unitary discharges of the vestibular nuclei to lateral tilt toward both directions. (*A*) Increased type. (*B*) Decreased type. (*C*) Reversed type I. (*D*) Reversed type II. *n*: Number of units.

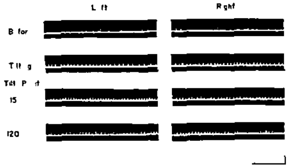


Fig 2 Effect of lateral tilt to both directions on unitary discharges of the vestibular nuclei (increased type) Calibration *Ordinate* 1 mV *abscissa* 0 5 sec

and 5) the refractory type in which the firing rate did not change significantly by lateral tilt to both directions As shown in Table I 10 (10 8%) 56 (60 2 %) 4 (4 3 %) 19 (20 4) and 4 (4 3 %) of 93 vestibular units exhibited these types respectively

Among the increased type of units 7 units showed an increase in frequency in response to lateral tilt to both directions and the other 3 units responded to either direction with a significant increase in the firing rate In the decreased type of units 50 units responded to lateral tilt to both directions with a significant decrease in the firing rate

Fig 1 shows the time-course of changes in mean firing rate of the unitary discharges recorded from the increased type of 7 units (*A*) the decreased type of 50 units (*B*) the reversed type I of 4 units (C) and the reversed type II of 19 units (D) when the head of the animal was tilted sideway through 30° and held in that position for 2 min The increased type and the reversed type II of the units showed the relatively lower frequency of the spontaneous discharges before lateral tilt while the decreased type and reversed type I of the units exhibited the higher frequency in the horizontal position In the increased type of units the firing rate was increasing progressively after the onset of lateral tilt in both directions and the increased discharges reached their peak effect of about 30/sec at 10 sec after attaining the inclination of 30 from the horizontal level Fig 2 illustrates a typical unit which produces an increase in the firing rate of discharges by lateral tilt to both the left and right The mean frequency of the discharges in the decreased type of units began to decrease immediately after the onset of lateral tilt to both directions until the maximal effect of 3 to 4/sec A typical example of the decreased type of unit is illustrated in Fig 3 The reversed type I and II of the units showed a transient increase in frequency after the onset of lateral tilt to both directions Thereafter the frequency of the discharges was reduced considerably by lateral tilt to either direction

Responses of the vestibular neurons to caloric stimulation

The responses to both lateral tilt and caloric stimulation in the same neuron were recorded from 31 single neurons of the vestibular nuclei As shown in Table II the responses of the vestibular unitary discharges to caloric stimulation with cold and warm water were classified into five types according to the method described in the previous paper (Matsuoka 1967) Two units of increased type (6 4°) showed an increase in frequency with both cold and warm stimulation and six units of decreased type (19 4) exhibited a decrease with both stimulation Eight units of reversed type I (25 8°) showed a decrease in frequency with the cold stimulation and an increase with the warm one and eleven units of

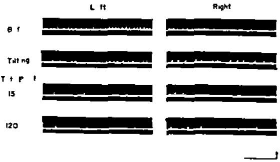


Fig 3 Effect of lateral tilt to both directions on unitary discharges of the vestibular nuclei (decreased type) Calibration *Ordinate* 1 mV *abscissa* 0 5 sec

Table II *Responses of the same single units of the vestibular nuclei to caloric stimulation and lateral tilt to the left in the intact cat*

Types	Caloric stim 30 C	Caloric stim 44 C	No of units	( )	In left tilt Responses	In left tilt No of units
Increased type	Increase	Increase	2	(6 4)	Decrease	2
Decreased type	Decrease	Decrease	6	(19 4)	Increase	3
					Decrease	3
Reversed type-I	Decrease	Increase	8	(25 8)	Increase	5
					Decrease	3
Reversed type-II	Increase	Decrease	11	(35 5)	Increase	1
					Decrease	9
					No change	1
Refractory type	No change	No change	4	(12 9)	Increase	3
					Decrease	1
Total			31			

reversed type II (35 5%) showed the reverse response to type I Four units of refractory type (12 9%) did not respond to either caloric stimulation The most dominant pattern of responses was the reversed type II

Eleven of the thirteen units of which the firing rate increases with the cold stimulation showed a significant decrease in frequency in response to lateral tilt to the left On the other hand eight of the fourteen units of which the firing rate decreased with the cold stimulation exhibited a significant increase in [illegible]y in response to lateral tilt to the left The other six of the latter units responded to the same inclination with a decrease in frequency Among the four units of refractory type to caloric stimulation three units showed an increase and the other one unit showed a decrease in frequency in response to lateral tilt to the left

Responses of the vestibular neurons in the hemilabyrinthectomized cats

When the cat awakened from barbiturate anesthesia after the right labyrinthectomy postural abnormalities head rotation and spontaneous nystagmus were observed The head of the animal was inclined to the right and rotated with the right ear downward The animal curled itself with the concavity of the body trunk toward the operated side and it could not stand in response to external stimuli The quick component of the spontaneous nystagmus was directed to the left Though the animal had restored walking behavior from 3 days after the operation it showed the ataxic locomotion and tendency to fall toward the operated side The head rotation and spontaneous nystagmus subsided gradually several days after the operation but the animal still showed the curving of the trunk and tended to lurch to the operated side Thirty to 40 days after the operation the animal had recovered almost normal posture and locomotion but tended to fall toward the right on running and jumping and the electronystagmographic examination confirmed the remaining presence of the spontaneous nystagmus toward the intact side

The mean firing rate of the spontaneous unitary discharges of the left vestibular nuclei on 7 14 21 and 42 days after the right labyrinthectomy were 11 1 ± 1 1 (S E) 8 2 ± 0 9 12 3 ± 1 7 and 17 9 ± 1 7/sec respectively (Fig 4) The lowest frequency was found at 14 days after the operation which was less than half of that (18 4/sec) in the intact animal Seven to 21 days after the labyrinthectomy many of the units showed the discharge frequencies from 0 1 to 15/sec and it was seldom found that the units with the spontaneous discharges above 30/sec in frequency

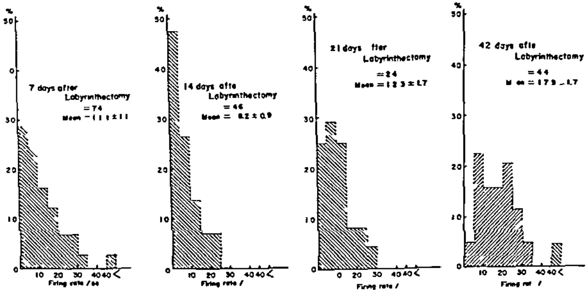


Fig 4 Distribution of the spontaneous unit discharges of the vestibular nuclei in the hemilabyrinthectomized cats *n* Number of units ± Standard Error

The mean firing rate and distribution of the spontaneous unitary discharges in the vestibular nuclei recovered to the control level at 42 days after the operation Table III shows the effects of lateral tilt to the left and right on the firing rate of unitary discharges of the left vestibular nuclei on 7 14 21 and 42 days after the right labyrinthectomy About half of the units showed a decrease in frequency in response to lateral tilt to both directions The vestibular units showing the opposite change in the firing rate according to the direction of inclination were in the range from 19 to 36° of units analysed In this respect the vestibular units in the contralateral labyrinthectomized animal did not behave differently to lateral tilt from those in the intact one Table IV shows the responses of the same single units to caloric stimulation of the ipsilateral middle ear cavity and lateral tilt to the left The decreased type reversed type I and II of the units in response to caloric stimulation with cold and warm were 16 2 and 6 of 24 units analysed respectively The duration of the responses to caloric stimulation was slightly prolonged in hemilabyrinth

Table III *Responses of the same single units of the vestibular nuclei to lateral tilt toward both directions in the hemilabyrinthectomized cat*

Types	No of units ( )			
	7 days after	14 days after	21 days after	42 days after
Increased type	4 (19 0)	5 (21 0)	1 (4 2)	3 (10 8)
Decreased type	13 (61 8)	12 (50 1)	11 (45 8)	14 (50 0)
Reversed type-I	3 (14 3)	3 (12.5)	4 (16 6)	2 (7 1)
Reversed type-II	1 (4 8)	4 (16 6)	7 (29 2)	7 (25 0)
Refractory type	0	0	1 (4 2)	2 (7 1)
Total	21	24	24	28

See Table I

Table IV *Responses of the same single units of the vestibular nuclei to caloric stimulation and lateral tilt to the left in the hemilabyrinthectomized cat (14–42 days after the operation)*

	Caloric stim				In left tilt	
Types	30 C	44 C	No of units	( )	Responses	No of units
Decreased type	Decrease	Decrease	16	(66 7)	Decrease	16
Reversed type-I	Decrease	Increase	2	(8 3)	Increase	1
					Decrease	1
Reversed type-II	Increase	Decrease	6	(25 0)	Decrease	6

ectomized animals It was 3 to 3 5 min at 14 days and 5 and 5 5 min at 42 days after the operation Only one unit showed an increase in frequency with lateral tilt to the left and the other 23 units responded to lateral tilt toward the same direction with a decrease in frequency after the contralateral labyrinthectomy

DISCUSSION

The peripheral vestibular organs consist of the semicircular canals and otoliths The receptor structures in the former organ are known to be activated by angular acceleration in a vertical transverse or anteroposterior axis and by caloric stimulation On the other hand the receptors of the otolith organs are known to be activated by gravity centrifugal force and linear acceleration Especially the utricular maculae are the organs of major importance in postural reflexes The observations of the thornback ray by Lowenstein & Roberts (1950) demonstrated that the receptors in the sensory endings of the otolith organs responded to lateral and fore and aft tilts Since the various labyrinthine reflexes from the body trunk could be excluded in the present *encephale isole* preparations the changes in the firing rate of the vestibular nuclei response to lateral tilt are likely to be caused by afferent impulses from the receptor endings in the otoliths Adrian (1943) and Peterson (1967) reported that many cells in the vestibular nuclei of anesthetized cats increased their firing rate when the ipsilateral side was tilted down and decreased their frequency when the contralateral side was tilted down In the present experiments on unanesthetized cats however the most dominant pattern of the response of the vestibular units to lateral tilt was decreased type (60 2%) in which the firing rate decreased in response to lateral tilt to both directions The reversed type I of response which showed an increase in frequency when the side of recording was tilted down and a decrease when that side was tilted up was found in only 4 (4 3%) of 93 units analysed The discrepancy between the mode of responses to lateral tilt in the anesthetized and unanesthetized animals suggested the central inhibitory mechanism to the vestibular nuclei i the latter preparations

The neuron activity of the unilateral ve tibular nuclei is more effectively analyzed b observing the responses to caloric stimulatic than to angular acceleration of the head whic activated the bilateral semicircular canals Th previous investigation by Matsuoka (1967) c the unanesthetized cats presented five differe types of response to caloric stimulation wit cold and warm water The increased type de creased type reversed type I and reverse type II in his reports were regarded as co responding with the type III IV II and of the responses to horizontal angular accelera tion classified by Dusing & Schafer (1958) an Shimazu & Precht (1965) according to th rule of Barany (1907) More than half of th vestibular units (61 3%) showed the chang in frequency to the cold and warm stimula

tion in the opposite direction and the most dominant pattern of the response was the reversed type II in which the firing rate increased with the cold stimulation and decreased with the warm one The ratio of each type of responses to caloric stimulation in the present experiments resembled that reported in the previous papers (Matsuoka 1967 1969)

Adrian (1943) proposed the presence of two main types of vestibular nuclei gravity-controlled and rotation-controlled types The former depended on the position of the head in space and the latter on angular acceleration or deceleration Among 31 units in the vestibular nuclei 12 units showed an increase in frequency with the cold stimulation but not with the ipsilateral tilt These units were regarded to belong to the above mentioned rotation-controlled type of units One unit increased the firing rate in response to both the cold stimulation and the ipsilateral tilt Eleven units showed an increase in frequency only with the ipsilateral tilt but not with the cold stimulation and these units were regarded to belong to the gravity-controlled type of units These findings agree with the results reported by Adrian (1943)

The anatomical and functional connection between the bilateral vestibular nuclei has already been reported by Gray (1926) Rasmussen (1932) Moruzzi & Pompeiano (1957) Shimazu & Precht (1966) and Matsuoka (1969) Since lateral tilt possibly affects the activities of the receptors in the bilateral otoliths the effects of lateral tilt were also studied on the vestibular unitary discharges in the *encephale isole* cats whose contralateral labyrinth had been completely destroyed previously The behavioral changes in the hemilabyrinthectomized cats such as postural abnormalities ataxic locomotion and spontaneous nystagmus were also similar in the course of development and manifestation of compensatory regulation with the observations by Fluur (1961) and Precht et al (1966) The firing rate of the spontaneous unitary discharges of the vestibular nuclei in the side of the intact labyrinth decreased to about half of that in the control animals 7 to 21 days after the labyrinthectomy but it recovered to the control level at 42 days after the operation It was demonstrated by Blomstrand et al (1966) in the hemilabyrinthectomized rabbits that the decrease of RNA content and the increase of succinoxidase activity in the vestibular nuclei of the healthy side at 15 days after the labyrinthectomy were restored to the previous level at 30 days after the operation. These biochemical changes caused by the contralateral labyrinthectomy seemed to coincide well in time-course with the change in frequency of the spontaneous unitary discharges caused by the same surgical procedures However the unilateral labyrinthectomy did not essentially affect the responses of the vestibular neurons in the healthy side to lateral tilt toward both directions. The responses of the vestibular units in the side of the operation to lateral tilt will be studied in further works

ZUSAMMENFASSUNG

Die Reaktionen der Einheitsentladungen des Vestibularkernes auf seitliche Neigung und Warmeanregung wurden an nicht anesthisierten „encepale isole Praparaten von intakten Katzen oder solchen mit Hemilabyrinthektomie untersucht Die Reaktionen von 93 Versuchsexemplaren auf seitliche Neigung wurden in 5 Gruppen unterteilt Zunehmender Typ abnehmender Typ Umkehrtyp-I Umkehrtyp-II und Refraktionstyp Die vorherrschende Reaktionsform auf die seitliche Neigung war der abnehmende Typ (60 2%) Kontralaterale Labyrinthektomie verminderte die Haufigkeit der spontanen Einheitsentladung im Vestibularkern wobei die verminderte Haufigkeit 42 Tage nach der Operation wieder das Kontrollniveau erreichte

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A METHOD FOR STUDIES OF THE MIDDLE EAR MECHANICS

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Abstract A method for studying the middle ear mechanics is described It is a further development of a method described by Ingelstedt et al (1967) The basic principle is the recording of the volume deviation of the tympanic membrane during and after changes of the ambient as well as the intratympanic pressure The method permits quantitative studies of the elastic properties of the tympanic membrane system during both static and dynamic pressure conditions The volume of the closed airfilled middle ear can be determined as can the tubal function during both static and dynamic conditions A new way to determine the middle ear pressure is presented

The physiology of the middle ear and the Eustachian tube is not completely understood and the clinician often faces problems connected with the middle ear In functional studies it is most important to realize that the methods used must not interfere with the physiological function of the ear In the present paper we have further developed a method basically described by Ingelstedt et al (1967) This method offers increased possibilities for quantitative studies of all the parameters involved in the mechanics of the middle ear These investigations are considered to be the fundamental basis for analysis of the mechanics of the middle ear in numerous pathological cases The present paper includes a report of the method used for recording of the normal tubal function under static as well as dynamic pressure conditions the elastic properties of the tympanic membrane the middle ear systems and the pressure in the middle ear

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The work is a part report in a series of papers on middle ear mechanics (Ingelstedt et al 1967 Elner et al 1971)

The method is based on recordings of the velocity of the movement of the ear drum and its volume deviation caused by pressure changes

P_{tm} the atmosphere pressure on the ground
P_m the pressure in the middle-ear
P_{ec} the pressure in the external ear canal
P_{tm} the pressure difference across the tympanic membrane i e $P_m - P_{ec}$
P_h the pressure in the pressure chamber i e the ambient pressure
P_{rh} the pressure in the rhinopharynx
$P_{ch_{V_{max}}}$ the ambient pressure at maximum drum movement
V_m the volume of the airfilled middle ear space
V_{tm} the volume deviation of the tympanic membrane in relation to its neutral position
V_m the volume of the mucous membrane lining the middle ear space
V_{diff} the gas volume which diffuses to or from the middle ear mucosa
V_t the gas volume passing the tube when open
V_{ar} the volume of the flowmeter system including the external ear canal up to the ear drum
V_r the volume of the reference flowmeter system
V_{tm} the air flow velocity through the resistor of the ear canal flowmeter caused by the volume deviation of the tympanic membrane
V_{ec} the air flow velocity through the resistor of the ear canal flowmeter caused by compression or expansion of the gas in the external ear canal and in the flowmeter system by changing the ambient pressure
V_{ar} the total flow velocity through the ear canal flowmeter $V_{tm} + V_{ec}$
V_{ref} the flow velocity through the resistor of the reference flowmeter caused by compression or expansion of gas in the reference system by changing the ambient pressure

Δ before the symbol indicates a change of the variable
Pressure is expressed in cm H_2O volume in microliter μl or milliliter ml and air flow velocity in μl/sec
P_m P_{ec} P_h and P_h are relative to ambient pressure on the ground

either in the middle ear or in the external ear canal In three following papers we will present results of investigations on the normal function of the Eustachian tube the middle ear pressure and the elastic properties of the tympanic mem brane system in 102 normal subjects

METHOD

An apparatus has been constructed (as shown in Fig 1) for the recording of the movement velocity of the tympanic membrane and its vol ume deviation caused by changes in the ambient pressure and in the middle ear pressure The apparatus is based on a principle described in 1967 by Ingelstedt Ivarsson & Jonson By this previous method the volume deviation of the tympanic membrane could only be produced by pressure changes in the middle ear ΔP_m while the ambient pressure P_{ch} was kept constant The apparatus consisted of a flowmeter which was airtightly connected to the external ear canal by a catheter system and a cuff The method did not permit recordings of the tym panic membrane caused by changes in the am bient pressure If for instance P_{ch} was increased the sum of two airflows was recorded one caused by the inward movement of the tympanic mem brane V_{tm} and another by the compression of ir within the external ear canal and the flow system V_{ec} For an isolated recording of volume deviation of the drum the flow V_e must be eliminated This elimination can be done by using another identical flowmeter system a reference system (Fig 1) This system is provided with a variable closed volume ar rangement so that the volume of the whole reference system can be adjusted exactly like the volume of the ear flowmeter system up to the tympanic membrane When this adjustment has been performed V_{ef} will be the same as V_e during the pressure change in the chamber By subtracting the flow signals from the two flow meters an isolated recording of the velocity of the movement of the drum V_{tm} is obtained The V_{tm} signal is then integrated and the volume deviation of the tympanic membrane is recorded ΔV_{tm} (Fig 1)

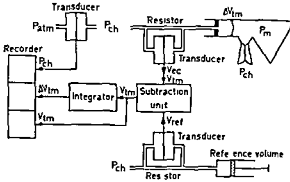


Fig 1 Block diagram of the apparatus for isolated re cording of the movement velocity of the ear drum V_{tm} and its volume deviation ΔV_{tm} For other symbols see page 1

Equipment

The method consists of following units

Pressure chamber

The investigations are performed in a pressure chamber in which the subject the investigator and the apparatus are enclosed The pressure in the chamber can be adjusted from $+50$ cm H_2O to -50 cm H O or vice versa Every pressure change takes 25 sec independently of the magni tude and is linear during 90% of the pressure change For further data see Ingelstedt et al (1967)

Cuff system

The catheter is fitted with a rubber disc and is airtightly placed in the inner bony part of the external auditory canal

Flowmeters

The ear canal flowmeter resistor and transducer (EMT 32 AB Elema Schonander Stockholm) The reference flowmeter resistor and transducer EMT 32 These flowmeters are identical

When an airflow passes the flowmeter it is recorded and converted to an electrical signal directly proportional to the flow velocity (Ingel stedt et al 1967) The transducers are balanced in respect to thermic zeroline drift which is mainly caused by temperature changes in the membrane of the transducers It is important to check this factor because the membranes are directly exposed to big temperature changes during the compression or expansion of gas in the systems

Pressure transducers

The pressure in the pressure chamber and the rhinopharynx are recorded with separate pressure transducers (EMT 35 AB Elema Schönander Stockholm)

Thermostat

To prevent any thermic effect on the electronics of the flowmeters these are mounted in an air tight thermostat container with a constant wall temperature of 18±0 01 C By keeping the container airtight the ambient pressure changes and thereby the temperature changes are prevented from affecting the flowmeters

Device for adjusting the reference volume

With the aid of an airtight syringe connected to the reference flowmeter system its volume can be varied Volume adjustment is performed by a servo system

Differential amplifier and integrator

The signals from both flowmeters i e the ear canal and reference system are subtracted and amplified The subtracted flow signal is integrated with respect to time and a volume signal is obtained

Recorders

The flow volume and pressure signals are amplified and recorded on a multichannel recorder (Mingograph 800 AB Elema Schonander Stockholm) and simultaneously on a tape recorder (Ampex FR 1300) The signals can be recorded on an xy recorder as pressure/volume or pressure/flow

Data of the Apparatus

Recorders

Multichannel recorder linearity ±1 % of 80 mm recorded deflection xy recorder linearity ±0 1 % of max recorded deflection

Pressure transducers EMT 35

Pressure range ±400 cm H O Linearity less than 2 within the pressure range

Ear and reference flow volume measuring devices

Differential pressure transducers EMT 32

Internal pressure chamber volume 2×0 4 ml Pressure range ±15 cm H O Displacement of the transducer membrane 10 μl/cm H_2O Hysteresis of the transducer membrane <0 1 % of the pressure measured Thermal zero deviation <0 0375 cm H O/°C

Flowmeter and catheter system

Resistance (resistor+catheter system) 6 25 × 10^{-3} cm H_2O/μl per sec of which the resistor 0 8 × 10^{-3} cm H_2O/μl per sec Volume (resistor+0 4 ml of EMT 32+catheter system) 3 ml Linear flow response 0-500 μl/sec Dynamic sinusoidal response flat over 0–11 cps Transient response (95 %) 20 msec

Ear and reference flowmeter systems have identical data and are tested separately For the test procedure for obtaining flow response sinusoidal and transient response see *Acta Otolaryng* Suppl 228 1967

The complete flow volume measuring apparatus

The zeroline drift at constant pressure in the pressure chamber ≤±0 25 μl/min Zeroline drift at repeated maximal pressure changes in the pressure chamber <±0 5 μl/min

These data have been obtained with the ear canal flowmeter system airtightly connected to a closed rigid copper container (volume 0 3 ml) thermostatized at 37 C The flowmeter systems were adjusted so that the air volumes were equal ($V_{ref}=V_{ea}$) during the analyses

Calibration

Flow volume measuring unit

The calibration of the gas volume passing the resistor is made before and after each experiment with the aid of an airtight microliter graded syringe The apparatus was tested by varying the velocity and the sliding range of the piston for volume corresponding 2 10 and 20 microliters up to a maximal air flow of 500 microliters/sec The accuracy of the volume calibration 1 μl ±5 %

Pressure measuring unit (EMT 35)

The pressure calibration was made before and after each experiment with 2 5 and 10 cm H O The accuracy of the pressure calibration 1 cm H_2O ±5%

During all calibrations the complete apparatus was used on the subjects The stability of the calibration is perfect during experiments lasting up to 5 hours

Model Experiment

The accuracy of the recording of the velocity of the drum movement and volume displacement at changing ambient pressure must first be studied This has been done by connecting the ear flowmeter to a middle ear model (Fig 1) where the drum consists of a rubber membrane with known volume pressure characteristic $V_{tm}=1\ 5 \times P_{tm}$ both under static and dynamic pressure conditions

Sources of error

(*a*) To be able to record the volume deviation of the rubber membrane separately at changing ambient pressure the volume of the two flow meter systems must be made equal $V_{ref}=V_a$ at $P_{tm}=0$ See volume balance page 6

(*b*) The movable drum disturbs the volume ance $V_{et}+V_{ea}$ during the pressure change influence of this factor on the recorded ume pressure characteristic is not more than 0 17 μl after changing the chamber pressure ±15 cm H O

(*c*) The registered volume pressure curve $\Delta V_{tm}=f(\Delta P_{ch})$ does not follow the same course at decrease and increase of the ambient pressure This hysteresis in the volume pressure recording amounts to 0 11 μl when the pressure changes at a rate of 2 cm H_2O/sec

The following factors contribute to the hystere sis and can be calculated from the data of the method

1 There is a pressure drop when gas flows through the flowmeter system because of its resistance In the ear flowmeter system this pres sure drop amounts to 0 06 cm H_2O i e the product of the resistance of the system and the air flow ($V_{ec}+V_{tm}$) There is thus a lag of the ear canal pressure in relation to the ambient pressure which contributes to 0 09 μl of the hysteresis as calculated from the volume pressure characteristic of the rubber membrane $V_{tm}=1\ 5 \times 0\ 06$ This is explained by the fact that the method records the volume deviation of the membrane as a function of the ambient pressure while the membrane is mobilized by the ear canal pressure

2 During the change of the chamber pressure the velocity of the airflow through the ear flow meter is higher than that through the reference flowmeter, $V_{tm}=3\ 0$ μl/sec because of the move ment of the rubber membrane The membranes in the two transducers will differ in deviation This difference in volume deviation is recorded as hysteresis and amounts to 0 024 μl and is calculated as the product of the volume displace ment of the EMT 32 membrane the resistance of the resistor and V_{tm}

The Influence of the Method on the Biological Recording

We have chosen two examples of tympanic membranes one normal and one flaccid Their volume pressure curves are assumed to be with out hysteresis under dynamic pressure condi tions

Theoretical prerequisite recording according to Fig 1 where the volumes of the flowmeter systems are balanced with the drum in its neutral position With the aid of the data obtained by the method and the volume pressure relationship of the tympanic membranes two volume pressure curves $\Delta V_{tm}=f(\Delta P_{ch})$ have been calculated when the chamber pressure is changed from +15 to −15 cm H_2O and the reverse with a velocity of 2 cm H_2O/sec This pressure change corre sponds to the maximal change used in studies of the volume pressure curves on living subjects (Fig 2) The hysteresis of the volume pressure recording varies and is maximal around the neutral position of the drum since it is propor tional to the velocity of the drum movement V_{tm}

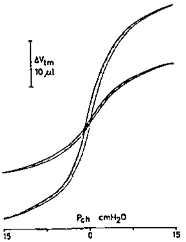


Fig 2 The ideal assumed volume pressure curve for a normal and flaccid tympanic membrane (interrupted curve) compared to the recorded one (solid curve) $\Delta V_{tm}=f(\Delta P_{h})$

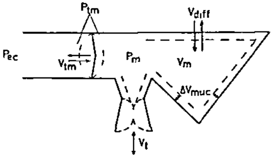


Fig 3 Middle ear with its different variables

THE MIDDLE EAR

The middle ear and its air cells constitute a system the normal function of which involves the interplay of several different variables (Fig 3) These variables are the closed airfilled space of the middle ear V_m the volume variation of the mucous membrane ΔV_{mu} the gas diffusion across the mucous membrane V_{diff} and the gas volume which passes the tube when opened V_t the pressure of the middle ear P_m and finally the pressure in the external ear canal P_{ec} All these factors are involved in the middle ear mechanics interact in different conditions and affect the pressure difference across the tympanic membrane P_{tm} whereby this membrane bulges outwards or inwards V_{tm} in relation to its neutral position

For the calculation of these variables by recording the volume deviation of the tympanic membrane the elastic characteristics of the tympanic membrane and of the middle ear systems must first be determined under static pressure conditions Ingelstedt et al (1967) The tympanic membrane system is defined as the tympanic membrane with the ossicles ligaments and muscles and its elastic characteristic is determined as the volume pressure relation $V_{tm}=f(P_{tm})$ The middle ear system is defined as the tympanic membrane system including the closed airfilled volume of the middle ear and the elastic characteristic of the middle ear system is determined as the volume pressure relation $V_{tm}=f(P\ )$ The diffusion of gases across the mucous membrane in the middle ear has been disregarded in this work As this process is very slow it does not affect the recordings during the different short experimental times used below

EXPERIMENTS ON MAN

For accurate isolated recording of the volume deviation of the tympanic membrane and the velocity of the drum movement the cuff fixation and the volume balance of the system must be kept constant during the whole experiment

Cuff fixation

The catheter with a rubber disc in the aural end is connected airtightly to the inner bony part of the external auditory canal and a further blocking is made with high vacuum grease on the outside of the rubber disc The diameter of the rubber disc is chosen individually for each subject and it is introduced under microscopic control The airtightness of the cuff is checked several times during each experiment The accuracy of the fixation is checked at the beginning and the end of the experiment in the following way the subjects are told to make maximal mandibular movements during which the recorded movement of the cuff should not exceed 0 2 μl A further check is provided by recording of the volume balance between the two flowmeters during the experiment

Volume balance procedures

Three different procedures are used depending on the capacity of the subjects to equilibrate the middle ear pressure

1 If the subject can equilibrate the middle ear pressure completely the neutral position of the tympanic membrane can be directly recorded (Fig 4*a*) If for example the chamber pressure decreases the tympanic membrane moves out wards This is recorded when there is a dif ference in volume between the two systems When the chamber pressure is constant the drum returns to its neutral position during degluti tions (Fig 4*b*) It is now possible to record the difference in gas volume evacuated from the systems (Fig 4*c*) The volume difference is calculated and the reference volume is directly corrected to volume balance $V_{ref} = V_a$ (Fig 4)

2 If the subject cannot equilibrate completely the neutral position of the tympanic membrane cannot be directly determined In such a case another well defined position of the tympanic membrane must be used If the subject produces a positive pressure in the middle ear by in creasing the pressure in the upper airways when the nose is clamped i e a Valsalva manoeuvre he will get a middle ear pressure of more than 30 cm H O and the tympanic membrane will bulge maximally outwards (Fig 5*a*) A small variation of the middle ear pressure at these high levels does not affect the drum because it has a very low elasticity in this position If the chamber pressure, P_{ch} is increased the drum moves inwards which can be recorded together with a possible difference in the gas volume con veyed to the systems Thereafter when the chamber pressure is constant a new Valsalva manoeuvre is made whereby the tympanic mem brane is once more forced maximally outwards At a state of balance between the two flowmeter systems the position of the tympanic membrane (Fig 5*b*) is recorded at the same level as that before the change of chamber pressure (Fig 5*a*) From this recording it is possible as in the example in Fig 4 to calculate the volume dif ference between the two flowmeter systems

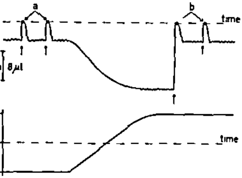


Fig 5 Recording procedure for calculation of a volume unbalance with the aid of the Valsalva manoeuvre In the example above there is a balance between the volume of the two systems Arrows mark Valsalva s manoeuvres

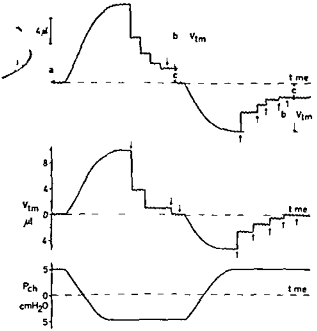


Fig 4 Procedure for calculation of volume balance In the upper half $V_{ar} \sim V_r$ Below the recording after adjustment to volume balance Arrows mark deglutitions

3 In the case of the very few subjects (4 %) who cannot produce a positive pressure in the middle ear by Valsalva s manoeuvre a different procedure must be used for the calculation of the volume balance First the chamber pressure is increased to +40 cm H_2O and the tympanic membrane is forced maximally inwards A small change of the pressure at this level does not further affect the position of the tympanic mem brane as it has a very low elasticity in this posi tion

Accuracy of the procedures

During the recording of the volume displacement of the drum during changes in the ambient pressure it is evident from the model experiments that the volume of the reference system must be the same as that of the ear canal system when the drum is in its neutral position This balancing is made according to procedure 1 and 2 as the drum moves approximately the same distance inwards and outwards when the chamber pressure changes If the balancing is made according to procedure 3 however the drum moves maximally inwards so a correction is made for the volume deviation in relation to the neutral position

On 10 subjects who can completely equilibrate the middle ear pressure all three types of volume balance procedures have been compared Procedure 1 and 2 give the same results Procedure 3 however give a volume unbalance up to 25 μl This means that the tympanic membrane when moved inwards by the overpressure in the chamber is not completely immobilized

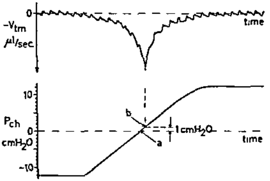


Fig 6 Recording procedures at indirect determination of the neutral position of the tympanic membrane Upper tracings indicate the velocity of the movement of the tympanic membrane when moving inwards ($-V_{tm}$) and below the tracing of the P_{ch} increasing (*a*) pressure in the ear canal after correction when the tympanic membrane is calculated to be in its neutral position (*b*) $P_{ch_{V_{max}}}$

Recordings and calculations of middle ear mechanics

I *Neutral position of the tympanic membrane*

The neutral position is defined as the position where the pressure difference across it is zero ($P_{tm}=0$) This position is determined according to two different procedures depending on the ability of equilibration

(*a*) *Direct determination* If the subject can completely equilibrate his middle ear pressure the neutral position can be recorded directly (Fig 4)

(*b*) *Indirect determination* On those who cannot completely equilibrate the middle ear pressure the neutral position of the drum is indirectly determined during a pressure increase in the chamber The location of the neutral position is calculated at the ambient pressure point where the velocity of the drum movement inwards is at its maximum $P_{ch_{V_{max}}}$ after a correction with -1 cm H_2O (Fig 6) This procedure for indirect determination of the neutral position has been obtained by comparison with the directly recorded neutral position on 61 subjects who could equilibrate completely Elner et al (1971*b*)

II *Direct determination of the volume pressure diagram of the tympanic membrane system under static pressure conditions*

The elastic characteristic of the tympanic membrane system can be defined in a volume pressure diagram as the volume deviation of the tympanic membrane V_{tm} a function of the pressure difference across it P_{tm} The origo in the diagram (Fig 7*a*) represents the neutral position of the tympanic membrane This volume pressure relation $V_{tm}=f(P_{tm})$ can only be recorded on subjects who can equilibrate completely The volume deviation of the tympanic membrane outwards or inwards in relation to its neutral position is recorded at different known pressure differences across the membrane The pressure in the external ear canal is kept constant with the aid of a catheter connected with the atmosphere The pressure change across the membrane only takes place from the middle ear when the subject swallows whereby over and underpressure is propagated quantitatively from the pressure chamber to the middle ear After each determina

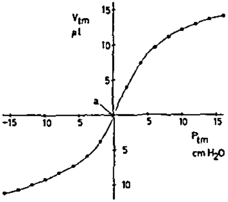


Fig 7 The static volume pressure diagram of the tympanic membrane system i e the volume deviation of the tympanic membrane in relation to its neutral position *a* as a function of the pressure difference across the tympanic membrane P_{tm} (+ V_{tm} outwards − V_{tm} inwards) The dots on the curve mark each single determination

tion it is checked that the tympanic membrane moves back to its original neutral position The recorded values are plotted in a diagram (Fig 7) describing the volume deviation of the tympanic membrane as a function of the pressure across it $V_{tm}=f(P_{tm})$ under static pressure conditions Ingelstedt et al (1967)

III *Direct determination of the volume pressure diagram of the middle ear system* *static pressure conditions*

elastic characteristic of the middle ear system is defined in a volume pressure diagram as the volume deviation of the tympanic membrane V_{tm} a function of the pressure in the external ear canal P_e when the tube is closed Origo in the diagram (Fig 8*a*) represents the neutral position of the tympanic membrane This volume pressure relation can only be recorded on subjects who can equilibrate completely The volume deviation of the tympanic membrane outwards and inwards in relation to the neutral position is recorded during stepwise increase or decrease of the ambient pressure when the tube is closed The recordings are made at constant pressure levels After each determination it is checked that the tympanic membrane during equilibration moves back to its original neutral position (Fig 4) The recorded volume pressure values are plotted in a diagram (Fig 8) describing the volume deviation of the tympanic membrane as a function of the pressure in the external ear canal $V_{tm}=f(P_{ec})$ under static pressure conditions

IV *Closed airfilled volume of the middle ear*

The closed airfilled volume behind an intact tympanic membrane can be assessed by calculation if we know the elastic characteristics of the tympanic membrane and the middle ear systems under static pressure conditions $V_{tm}=f(P_{tm})$ and $V_{tm}=f(P_{ec})$ (see Ingelstedt et al 1967)

V *Direct determination of the volume pressure curve of the middle ear system during dynamic pressure conditions*

The volume pressure curve of the middle ear systems $\Delta V_{tm}=f(\Delta P_{ch})$ can be directly recorded independently of the subject s ability to equilibrate during the change of chamber pressure i e under dynamic pressure conditions In contrast to the volume pressure relation of the middle ear system during static pressure conditions there is a hysteresis in the volume pressure curve as appears from Fig 9 i e the tympanic membrane does not follow the same volume pressure relation at decrease and increase of the chamber pressure As is evident from the

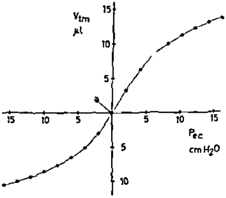


Fig 8 Static volume pressure diagram of the middle ear system i e the volume deviation of the tympanic membrane as a function of the pressure in the external ear canal in relation to the neutral position of the tympanic membrane $V_{tm}=f(P_{ec})$ Neutral position *a* The dots on the curve mark each single determination

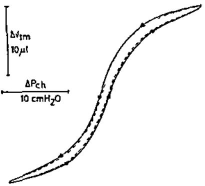


Fig 9 The dynamic volume pressure curve of the middle ear system The solid curve corresponds to the directly recorded $\Delta V_{tm} = f(\Delta P_{ch})$ and the interrupted curve after correction for the hysteresis of the method $\Delta V_{tm} = f(\Delta P_{c})$ Upward arrows illustrate the volume deviation of the tympanic membrane outwards during decrease of the external ear canal pressure Downward arrows correspond to deviation during increase of the pressure in the external earcanal

recordings on Fig 9 the hysteresis of the middle ear system curve is much greater than that of the method (Fig 2) when a tympanic membrane of normal mobility is studied

With the aid of the dynamic volume pressure curves of the middle ear system $\Delta V_{tm} = f(\Delta P_{c})$ recorded at different pressures a corresponding static volume pressure curve can be constructed (Elner et al 1971*c*) The neutral position is determined indirectly and a volume pressure diagram is constructed around this point describing the volume deviation of the drum as a function of the pressure in the ear canal $V_{tm} = f(P\ )$ under static pressure conditions This procedure for determination of the elastic properties of the middle ear system is the only one available in cases with a reduced equilibration capacity

VI *Pressure difference across the tympanic membrane*

The pressure difference across the tympanic membrane P_{tm} is defined as the middle ear pressure in relation to the ambient pressure i e chamber pressure P_{h} This difference can be determined by two different procedures depending on the ability of the subject to equilibrate

(*a*) *Direct determination* On subjects who can equilibrate completely the volume deviation of the tympanic membrane from its neutral position can be recorded i e the actual volume position of the tympanic membrane before equilibration With the aid of this value the pressure across the tympanic membrane can be calculated from the volume pressure diagram of the tympanic membrane system $V_{tm} = f(P_{tm})$

(*b*) *Indirect determination* On subjects who cannot equilibrate completely the neutral position of the tympanic membrane is determined indirectly according to the principle on page 7 When the tympanic membrane is in its neutral position the pressure on the inside of the drum is the same as the recorded pressure on the outside $P_{eo} = P_{m}$ This pressure in the external ear canal does not however correspond to the original pressure in the middle ear During the procedure of determination the tympanic membrane makes a volume deviation which changes the closed airfilled volume and thereby the pressure of the middle ear The original pressure in the middle ear i e the pressure difference across the tympanic membrane is calculated after correction for the recorded volume deviation of the tympanic membrane assuming a mean volume of the closed airfilled space of the middle ear of 6 ml Elner et al (1971*b*)

VII *Pressure regulatory capacity of the Eustachian tube under static pressure conditions*

By changing the ambient pressure to constant levels a pressure difference across the drum is created P_{tm} i e a relative over or underpressure is produced in the middle ear in relation to the ambient pressure P_{h} The tympanic membrane then deviates outwards or inwards from its neutral position Thereafter the subject equilibrates and the drum moves back towards its neutral position which expresses the pressure regulating capacity of the Eustachian tube (Fig 4) The pressure equilibration across the tympanic membrane is calculated with the aid of the volume pressure diagram of the tympanic membrane system $V_{tm} = f(P_{tm})$ In case of a reduced tubal function an indirect determination

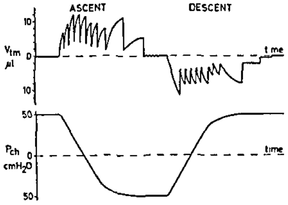


Fig 10 Equilibration of dynamic relative over and under pressure in the middle ear i e during ascent and descent by maximal frequency of deglutitions Upper tracing the recorded volume deviation of the tympanic membrane in relation to its neutral position Lower tracing the pressure change in the pressure chamber

of the neutral position of the tympanic membrane has to be performed first according to the principle presented on page 7 Thereafter the chamber pressure is changed to a new constant level the subject is told to equilibrate and a possible volume deviation of the tympanic membrane towards the neutral position is recorded The experiment is finished by making an indirect determination of the pressure difference across the tympanic membrane after the equilibrations according to the procedure on page 9 A possible residual pressure P_{tm} expresses the degree of reduction of the tubal function

VIII *Pressure regulatory capacity of the Eustachian tube under dynamic pressure conditions*

The volume deviation of the tympanic membrane in relation to its neutral position can be recorded at fast changes of both chamber pressure and middle ear pressure on subjects who can equilibrate completely This method thus makes it possible to make direct quantitative studies of the tube s capacity to equilibrate during the changes of P_{ch} by recording the volume deviation of the tympanic membrane during the wh pressure change period ascent or descen both when the tube is open and closed Fig 1 The mean deviation of the tympanic membra from its neutral position during a whole pressu change period can be calculated by planimet and the mean pressure across the tympa membrane can be obtained from the volum pressure diagram of the tympanic membra system $V_{tm}=f(P_{tm})$ This mean pressure m be a quantitative measure of the pressure r gulating capacity of the Eustachian tube duri such advanced conditions as simulated ascen and descent

ZUSAMMENFASSUNG

Beschrieben wurde eine Methode zur Untersuchung d Funktionsmechanismen im Mittelohr die in einer We terentwicklung einer von Ingelstedt et al (1967) b schriebenen Methode besteht Im Prinzip werden Vol menveranderungen des Trommelfells vor und nac Druckveranderungen in der Umgebung bzw in d Paukenhöhle registriert Mit der beschriebenen Methoc konnen die elastischen Eigenschaften des Trommelfel systems und die Tubenfunktion unter statischen ur dynamischen Druckverhaltnissen sowie auch das Volume des geschlossenen luftgefullten Mittelohres bestimn werden Ausserdem wird eine neue Weise zur Bestimmun des Druckes im Mittelohr beschrieben

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A COMPARATIVE ANALYSIS OF THE AUDIOVISUAL AUDITIVE AND VISUAL PERCEPTION OF SPEECH

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From The State Hearing Rehabilitation Centre Copenhagen Denmark

(Received February 19 1971)

Abstract This is first part of a long term study According to examinations of 30 subjects in the age groups 20 50 and 70 years all with normal hearing perception of speech in noise was found to deteriorate with age whether determined on the basis of audition alone or on audition+vision The capacity to perceive purely visual speech was found to be least satisfactory among subjects in the age group 70 years A comparison of the purely visual perception of speech and the audiovisual perception indicated that there is some correlation between the two Audiovisual results are more reliably reproduced than the auditive ones thereby indicating that the selection of hearing aids should be incorporated into the audiovisual test situation in noise With a view to the further advances in the testing of audiovisual speech perception the requirement must be an elaboration of a stimulus word material which makes equal allowance for the auditive the audiovisual and th visual perception

It is common courtesy to look at the person to whom you are speaking the virtue involved in this phrase may often be a requirement Vision is such an essential factor in linguistic communication that it may be reasonable to characterize normal perception of speech as a bisensoric phenomenon In the field of audiology however the component based upon perception via the ear has attracted the main interest This may suffice for audiological diagnostics but certainly not for audiological rehabilitation Vision constitutes an important factor in speech perception for which reason the attention should be concentrated on the interplay of audition and vision the aim must be to elaborate clinically applicable methods which will indicate a realistic impression of the communicative powers of an individual with defective hearing

So far the interplay of audition and vision has been the object of study on only a few occasions O Neill (1954) and Sumby & Pollack (1954) demonstrated the marked improvement in perception if the speaking persons were visualized during the audiovisual test The improvement was found to be proportionate to the signal to noise ratio being most marked when conditions were least favourable to the listeners According to Erber (1969) the auditional power of subjects might be exploited even under extremely adverse signal to noise ratios if only the speaking person might be visualized at the same time

In a preliminary report Ewertsen et al (1970) reported the results of 100 hard of hearing subjects tested by means of short sentences presented visually audiovisually and through hearing alone at various levels around their speech reception thresholds in the quiet There was a fairly good correlation between the subjects capacity for lip-reading and the permissible reduction of the presentation level of the sentences still giving complete comprehension However we were faced with so many problems that we decided to begin a long term study of which this is the first part

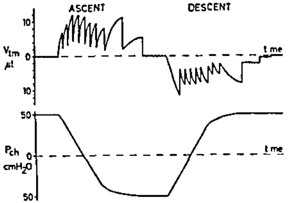


Fig 10 Equilibration of dynamic relative over and under pressure in the middle ear i e during ascent and descent" by maximal frequency of deglutitions Upper tracing the recorded volume deviation of the tympanic membrane in relation to its neutral position Lower tracing the pressure change in the pressure chamber

of the neutral position of the tympanic membrane has to be performed first according to the principle presented on page 7 Thereafter the chamber pressure is changed to a new constant level the subject is told to equilibrate and a possible volume deviation of the tympanic membrane towards the neutral position is recorded The experiment is finished by making an indirect determination of the pressure difference across the tympanic membrane after the equilibrations according to the procedure on page 9 A possible residual pressure P_{tm} expresses the degree of reduction of the tubal function

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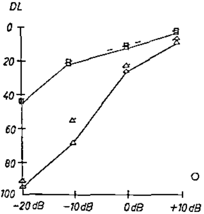


Fig 2 The test re test effect with a single person is illustrated Δ——Δ discrimination loss (percent) measured by hearing alone at the first examination under the four indicated signal to-noise ratios Δ---Δ corresponding results at the second examination □——□ discrimination loss (percent) measured audiovisually at the first examination □---□ corresponding result at the second examination ○ (lower right) visual discrimination loss (percent) at the first examination discrimination loss at the second examination. *Abscissa* signal to-noise ratio *ordinate* discrimination loss

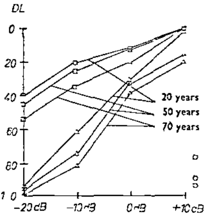


Fig 3 The curves show the average results at the first examination of 30 persons with normal hearing 10 in each of three age groups Δ——Δ results of a pure auditive presentation at various signal to-noise ratios □——□ results of the combined audiovisual perception ○ (lower right corner) average discrimination at purely visual presentation *Abscissa* signal to noise ratio *ordinate* discrimination loss

The tests were monaural and it was at the option of the subject whether he wanted to have the right or the left ear tested Usually the telephone-ear was preferred Subjects were retested after intervals of from 1 to 2 weeks By way of example the findings in a single subject are illustrated in Fig 2 This illustration depicts the ratio of the purely auditive to audiovisual speech perception at various signal to noise ratios together with the visual speech perception of 25 test words Finally the reproducibility of findings in this one man test is recorded It appears from Fig 2 that the reproducibility of all three parameters was satisfactory in this test of a typical subject His proficiency in lip-reading (circles in the bottom right hand corner) i e his perception of purely visual speech is almost nil However when you consider the results obtained under adverse noise conditions viz when the signal is weaker by 20 dB than the noise his proficiency in lip-reading is still of value Thus the audiovisual speech perception of this subject is about 50% in contrast to his auditive proficiency which under the same conditions is almost nil

RESULTS

The findings obtained in the series of 30 subjects are calculated as the average values obtained in each of the three age groups cf Fig 3 in which features are seen to be the same as those in Fig 2

At a signal to noise ratio of −20 dB the purely auditive tests showed total masking of speech while about 50 of the test words were perceived during the audiovisual tests The latter phenomenon is assumed to be attributable to the role of vision According to the results obtained in tests of the purely visual speech perception however the discrimination loss was very large for which reason the above assumption may not give the true explanation Thus the fairly satisfactory audiovisual speech perception at a signal to noise ratio of −20 dB must be due to the close interplay of vision and audition as demonstrated by Erber (1969) The results recorded in Fig 3 provide further evidence that the faculty of speech perception whether auditive or audiovisual seem to deteriorate parallel with advancing age Even the faculty of perceiving visual speech deteriorates parallel with age

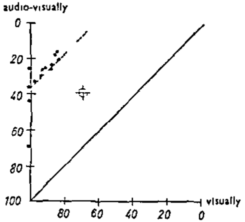


Fig 4 At the signal/noise ratio of −10 dB the results of audiovisual speech perception (*ordinate*) at the 1st examination of 30 normal hearing subjects have been related to the purely visual perception (*abscissa*) The numbers indicate the percentage of misunderstandings of 25 PB words

The audiovisual and the visual speech perception are correlated in a dot diagram (Fig 4) The diagram shows two aspects

1 There is a clear correlation between the two ways of perception

2 There is a clear difference between the two levels of perception because there are about 60° more misunderstandings in the visual than the audiovisual situation

DISCUSSION

It appears from Figs 2 and 3 that speech perception is optimal in the audiovisual test situation under poor signal to noise ratios The influence of vision on speech perception is obvious under conditions unfavourable to the listener thus confirming the findings obtained by the previously mentioned authors

According to the results obtained at tests 1 and 2 (Fig 2) the reproducibility of the audiovisual findings was found to be optimal in all 30 subjects the scatter of results obtained by auditive determinations being wider especially at a signal to noise ratio of 10 dB The fact that the precision with which audiovisual replies are reproduced is superior to that of the auditive replies may be of significance when hearing aids are to be selected and fitted Thus selection and fitting must not take place in the absence of noise It should be done rather under critical conditions for instance at signal to noise ratios of 10 dB Favourable changes in the amplification may be reflected in the improved audiovisual perception of speech Furthermore, it can hardly be doubted that training in hearing or lip reading exerts a recordable influence on the speech perception

In audiometric routine tests which usually are of purely auditive nature vision may play a distracting role in that the subject to be tested may catch sight of' certain objects in the test room his attention thus being diverted from the test per se Accordingly the aim should be to establish an audiovisual test situation because it reflects the common everyday speech situation Besides it must be ascertained that the speech perception is of bisensoric character involving vision as well as audition

The test words used in the present study were balanced auditively but not audiovisually If the audiovisual problems are to be further defined and elaborated it will be necessary to have a stimulus word material composed which is balanced auditively audiovisually and visually

ZUSAMMENFASSUNG

Dies ist der erste Teil einer grosseren Studienaufgabe In Verbindung mit den Untersuchungen von 30 Personen in den Altersgruppen 20 50 und 70 Jahren — alle mit normalem Gehor — wurde festgestellt dass die Sprachauffassung im Gerausch mit zunehmendem Alter abnahm ob es auf Grund des Gehors allein oder auf Gehor und Sehkraft bestimmt wurde Die Fahi keit rein visuelle Sprache aufzufassen fand man a schlechtesten unter Personen in der Altersgruppe vc 70 Jahren Ein Vergleich zwischen der rein visuel (Sprachauffassung und der audio-visuellen Auffassui zeigt dass eine Verbindung zwischen den beid(besteht Audio visuelle Ergebnisse sind mehr zuve lassig reproduzierbar als di auditiven wodurch ma feststellen kann dass die Wahl von Horgeraten in d(audio-visuellen Untersuchungssituation im Gerausc stattfinden muss Im Hinblick auf die weiteren For schritte in den Versuchen mit audio-visueller Sprac! auffassung muss es gefordert werden eine Bea

beitung des Wortmaterials zu machen die gleich grosse Rücksicht auf die auditive die audio-visuelle und die visuelle Auffassung nimmt

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MIDDLE EAR EPITHELIUM IN THE PRESENCE OF EXPERIMENTAL EFFUSION

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(Received November 30 1970)

Abstract Serous effusions were experimentally induced in squirrel monkeys 24 hours after obstruction of their Eustachian tubes with fine silicone rubber sponge material Electronmicroscopic study of the earliest epithelial changes of the mucoperiosteum revealed (*a*) evidence of fluid flooding widened intercellular spaces (*b*) deformation of non ciliated and basal cells with cytoplasmic changes while ciliated cells maintained a near normal appearance (*c*) ruptures of the basement membrane allowing fluid and cellular passage from the subepithelial to the epithelial layer

These observations suggest that the serous fluid in this model resulted from transudation of vessels rather than active cellular secretion

It is of interest to consider the pathogenesis of the various chronic inflammatory diseases of the Eustachian tube, middle ear and mastoid After doing so one is impressed by the common etiological factors suggesting close relationships between the various types

Using experimentally induced serous effusions in the monkey as a model the purpose of this study is to report the earliest electron microscopic changes of the mucoperiostium with special attention to the epithelial cells

METHOD AND MATERIALS

The Eustachian tubes of four healthy squirrel monkeys weighing between 500–1 000 g were obliterated through the pharyngeal orifice with fine grade silicone sponge after a midline incision of the soft palate The incision in the soft palate was sutured and antibiotics (benzothine, penicillin G 300 000 units per animal) were injected to protect against secondary invaders Twenty four hours after surgery serous fluid in the middle ear was confirmed in each animal through an incision in the tympanic membrane Both the middle ear and mastoid cavities were perfused in vivo through a puncture of the mastoid with 1 % osmium tetroxide solution buffered by phosphate pH 7 4 After decapitation the temporal bones were placed in the same fixative for 2 hours The mucoperiostium was removed while still in the fixative After fixation the tissue was dehydrated in a series of graded alcohols embedded in Epon 812 sectioned with an LKB ultratome stained with Auranyl acetate lead citrate and examined with an RCA EMU 3G microscope These findings were then compared with normal findings in the squirrel monkey (Fig 1)

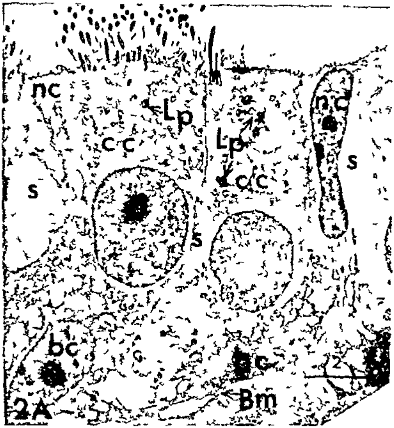
RESULTS

The most remarkable finding was the presence of widened extracellular spaces in the epithelial layer compressing the three types of epithelial cells The deformation of cells was much more noticeable in non ciliated cells and basal cells than in ciliated cells (Fig 2)

This investigation was supported by the National Institutes of Health Public Health Service Research grant NB07623



Fig 1 Normal middle ear epithelium of monkey (*A*) Ciliated cell area (× 4 450) (*B*) Nonciliated cell area (× 9 800) Note the width of the intercellular space and the similarity of appearance of cytoplasm of ciliated non ciliated and basal cells


nc
Lp
cc
Lp
cc
nc
s
s
s
bc
bc
2
Bm
2A

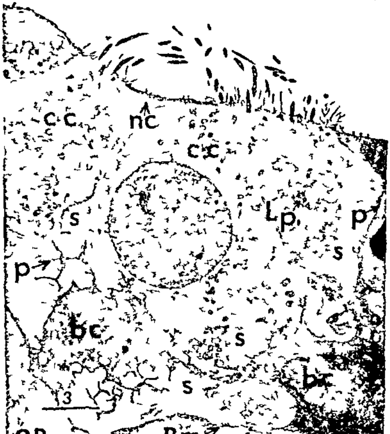

cc
nc
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s
Lp
p
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p
bc
s
bc
s
3
2B
Bm



Fig 2 Middle ear epithelium after formation of serous fluid Widened intercellular spaces are filled with serum Non ciliated and basal cells are severely deformed and have a dark appearing cytoplasm Ciliated cell cytoplasm is nearly normal in appearance (*A*) An increased number of lipid inclusions with pigment bodies are seen in the ciliated cell cytoplasm (×5 035) (*B*) Non ciliated cells and basal cells are markedly compressed by intercellular fluid The widened intercellular spaces were bridged by many finger like processes extending from these cells (×4 450) (*C*) Compare with Fig 1 *B* (normal case) Non-ciliated cell cytoplasm appears darker due to the increased number of ribosomes and an increased electron density of the ground substances (×8 000) *nc* non-ciliated cell *cc* ciliated cell *bc* basal cell *Bm* basement membrane *Lp* lipid inclusion *p* finger like process of epithelial cell *s* intercellular space *J* junctional complex

The wide extracellular spaces contained an amorphous substance characteristic of serum as seen in blood vessels Enlargement of the extracellular spaces varied from one animal to an other and also from one site to another in the same ear The wider extracellular spaces were bridged by many extensions of finger like processes of epithelial cells especially basal cells (Fig 2 *B*)

The basement membrane was usually seen immediately beneath the epithelial cells Rupture of the basement membrane was present in certain areas allowing direct communication between the sub-epithelial layer and the intercellular spaces of the epithelial layer (Figs 3 4)

In the wider intercellular spaces occasional wandering cells such as leucocytes fibroblasts or macrophages infiltrated through performations of the basement membrane from the sub-epithelial space (Fig 3)

In addition to deformation of epithelial cells

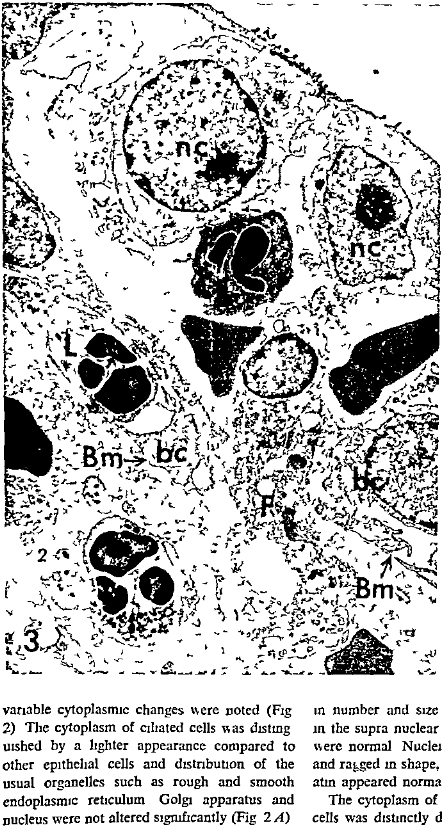


Fig 3 A wandering cell is breaking through the basement membrane to enter the epithelial layer Leucocytes and erythrocytes are seen in the intercellular spaces (× 6 500) *F* Fibroblast *L* leucocyte *E* erythrocyte *nc* non-ciliated cell *bc* basal cell *Bm* basement membrane

variable cytoplasmic changes were noted (Fig 2) The cytoplasm of ciliated cells was distinguished by a lighter appearance compared to other epithelial cells and distribution of the usual organelles such as rough and smooth endoplasmic reticulum Golgi apparatus and nucleus were not altered significantly (Fig 2 *A*) Osmiophilic lipid inclusions with pigment bodies and multivesicular bodies were increased in number and size and were usually located in the supra nuclear region (Fig 2 *A* 6) Cilia were normal Nuclei were sometimes irregular and ragged in shape, but distribution of chromatin appeared normal

The cytoplasm of non-ciliated cells or basal cells was distinctly dark in appearance due to an increased number of free ribosomes fine fibers and also an increased electron density

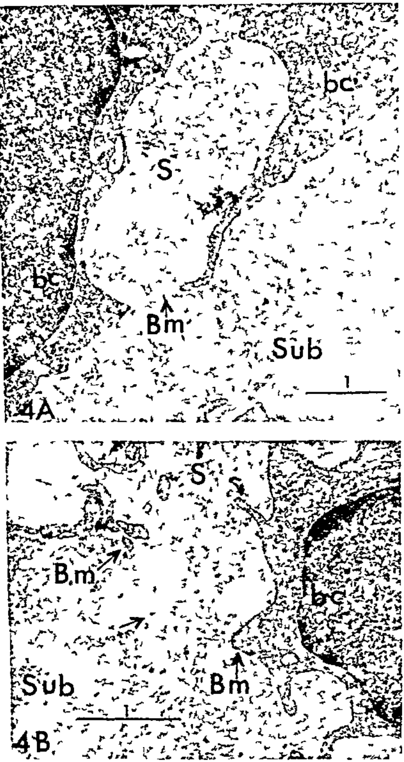


Fig 4 The basement membrane is in contact with the widened intercellular spaces of epithelial layer (*A*) (×21 000) Occasionally a rupture (*arrow*) of this membrane was seen (*B*) (×27 000) *Bm* Basement membrane *bc* basal cell *S* intercellular spa subepithelial layer

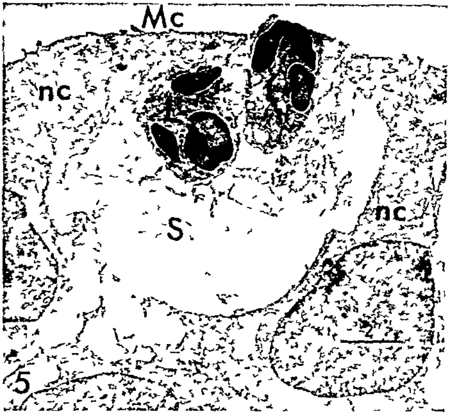


Fig 5 Two leucocytes are seen breaking through the junctional complex between epithelial cells (×7 100) *nc* non-ciliated cell *L* leucocyte *S* inter cellular space *Bm* basement membrane *Mc* middle ear cavity

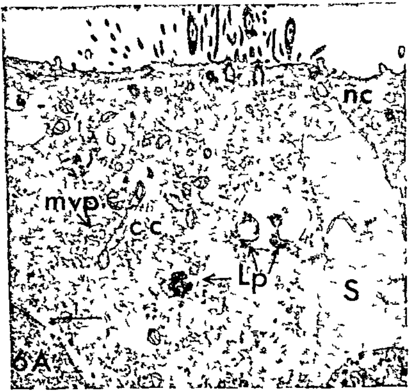
of ground substance (Fig 2 *C*) Osmiophilic lipid inclusions with pigment bodies were sometimes found less frequently than in ciliated cells (Fig 6 *B*) Deformation of these cells was more severe and multiple irregular finger like processes were seen extending into intercellular spaces Some of these processes were closely apposed and sometimes desmosomes were formed but many of the processes were separated from each other by wider intercellular spaces (Fig 2 *B*) Pinocytotic vesicles were easily found in normal basal cells but seemed to be reduced in number in some of these cases

DISCUSSION

Several theories regarding the pathogenesis of serous otitis media have been offered The most widely accepted theory which was proposed by Politzer (1883) is based upon the absorption of oxygen from the isolated volume of air in the middle ear cavity The mucous membrane lining the middle ear cavity begins to show hyperaemic ex vacuo which is sometimes accompanied by hydrops ex vacuo due to negative pressure relative to atmospheric pressure Opposition to this theory has arisen because the tube is often patent in human cases of chronic serous otitis media (Zollner 1935) Other reports have suggested that the tube is in some way responsible for this condition (Proud & Odoi 1970) (Buckingham & Ferrer 1966) Holmgren (1940) and Van Dishoeck (1941) showed that the maximum negative pressure in the middle ear resulting from Eustachian tube obstruction is 50 cm of water

Another proposed theory suggests that the effusion of the middle ear is due to lymphatic and/or venous stasis (Robinson 1942) A third theory is that an initial infection in the middle ear mucosa is responsible for serous otitis media. Allergy has also been clinically implicated as an etiological factor

From this study it was apparent that fluid



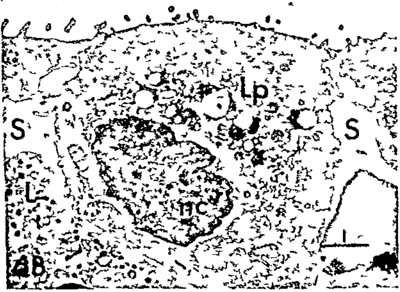


Fig. 6. Lipid inclusions are seen in ciliated cell (*A*) (× 12 000) and non-ciliated cell (*B*) (× 11 000). Often these inclusions seem to be bound by a single limiting membrane. They contained single or multiple oval lipid globules with numerous smaller electron-dense granules. Such pigment bodies are not uniform. *cc* ciliated cell. *nc* non-ciliated cell. *Lp* lipid inclusion. *mvp* multi vesicular body. *L* leucocyte. *S* inter cellular space.

greatly distended the extracellular spaces in the epithelial layer This fluid from its appearance probably contains protein or mucopolysaccharides similar to serum

Careful observations of the mucous membrane revealed extremely few secretory structures (granules) in normal or pathological specimens No glands were seen It is obvious that these few secretory granules could not be responsible for the serous fluid which filled the middle ear cavity The fluid must have originated from the fluid seen in the extracellular spaces of the epithelial layer

In addition leucocytes or erythrocytes were seen within the subepithelial and epithelial layers They were also seen breaking through the basement membrane of the epithelial layer suggesting the origin to be from subepithelial blood vessels Other evidence of vessel permeability in these animals with induced serous effusions comes from studies of capillary labeling with colloidal carbon particles (Hiraide & Paparella, 1970)

In the cytoplasm of mainly ciliated cells the increased number and size of osmiophilic lipid inclusions with pigment bodies was noted They resembled lipofuscin granules which are found in the cytoplasm of many types of cells (Frank Christensen 1968 Goldfischer & Bernstein 1969) It has been confirmed that lipofuscin granules are lysosomes because of their fine structure and acid phosphatase activity (Essner & Novikoff 1960) It is thought that they are mainly the undigested residue of lysosomal hydrolysis (Frank & Christensen 1968 Goldfischer & Bernstein 1969)

ACKNOWLEDGMENT

We would like to acknowledge Miss Pat Schachern Mrs Kathy Lauhala and Miss Carolyn Sutherland for their technical assistance

ZUSAMMENFASSUNG

Als Folgeerscheinung der experimentellen Obstruktion der Tuba Eustachii mit feinem Silikongummischwamm wurden nach 24 Stunden im Mittelohr des Totenkopfäffchens (Saimiri sciurea) systematisch seröse Flüssigkeitansammlungen gefunden. Die elektronmikroskopische Untersuchung der frühesten Epithelveränderungen im Mukoperiosteum in diesen Tieren zeigte a) stark ausgebreitete und mit Flüssigkeit prall gefüllte interzellulare Räume b) Deformationen der nicht Flimmer tragenden Zellen und der basalen Zellen begleitet von Veränderungen des Cytoplasmas Die Flimmerzellen erschienen kaum verändert c) Zerreissungen der Basalmembran erlaubten Flüssigkeit und Zellen freien Durchtritt vom Bindegewebe ins Epithel

Es ist sehr wahrscheinlich dass die angesammelte seröse Flüssigkeit im vorliegenden Falle ein Transudat darstellt.

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CHRONIC FRONTAL SINUSITIS TREATED BY AUTOGENOUS OSTEOPLASTY

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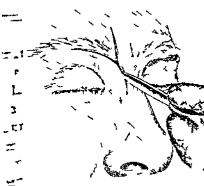
(Received December 15 1970)

Abstract An operative method for treating chronic frontal sinusitis is described The method involves ablation of the frontal sinus by autogenous osteoplasty through a curved incision from the ridge of the nose to the underside of the eyebrow with an auxiliary incision up toward the forehead The anterior wall of the frontal sinus is removed by drilling and the mucosa of the frontal sinus is meticulously withdrawn The upper opening of the nasofrontal duct is hermetically sealed with a peg of bone taken from the iliac crest The whole frontal sinus is then filled with spongiosa from the iliac crest The anterior wall of the frontal sinus is reconstructed to its normal configuration with mosaic plastics of cortical bone The incision is closed without drainage and dressed with a compression bandage This method has been used in operations on 11 patients in the years 1965-70 with excellent results

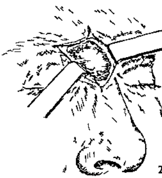
Chronic frontal sinusitis and its treatment was mentioned in reports hundreds of years ago At that time the ophthalmologists and surgeons believed in trephination of the frontal sinuses This therapy was used in cases of empyema in the frontal sinus with fistulation outward In the operation the fistula was widened to let the pus flow out freely from the sinus Much later Beck (1916) published a report on a similar method the so-called Beck trephination which is still found to be satisfactory The first scientific publication describing the treatment of a chronically infected frontal sinus was made by Ogston in 1884 As early as 1895 Kuhnt said that the only radical therapy in frontal sinus empyema is the removal of the mucosa and elimination of the sinus by removing its anterior wall In 1898 Schenke described Riedel s operation aiming at the complete elimination of the frontal sinus He emphasized particularly that all frontal sinus walls with the exception of the posterior wall had to be taken away The mucosa of the sinus also had to be meticulously removed The skin of the forehead together with the soft tissues subsequently collapses into the sinus against the posterior wall A great disadvantage of the method is however that it leaves a cosmetically ugly scar which is not easy to repair even by plastic surgery Killian (1903) praised the unrivalled radicalism of Riedel s method but admitted that the accompanying cosmetic defect heavily restricts its use At the same time Killian presented a new method of operation which gave a beautiful cosmetic result. He removed the mucosa and the majority of the anterior and lateral walls of the frontal sinus leaving a bridge of the anterior and lateral walls This bridge prevented the soft tissues of the forehead from collapsing into the frontal sinus and no depression developed after operation The wound usually healed *per primam* The rhinologic world was enthusiastic Disappointment followed after a few years Reports were published on relapses intracranial complications and fatalities Hajek (1936) reported relapses 15 and 17 years postoperatively The causative factor was that the infection remained dormant in the dead space left by the operation After

Killian s method had proved a failure, the rhinologists began to devote attention to the opening and enlargement of the nasofrontal duct Halle (1915) performed a transnasal operation which however proved to be technically difficult and even dangerous (Boenninghaus 1928 Marx, 1949) The extranasal route to the frontal sinus proved to be less dangerous and there are several methods of operation based on this principle (Jansen 1894 Ritter 1906 Barany 1924, Seiffert 1947 Uffenorde 1952) Relapses after these operations were also numerous Hajek wrote in 1936 that during his 45 years of work the treatment of chronic frontal sinusitis had given him much trouble and work but little satisfaction Walsh (1943) tried to discover why the results were poor He experimented on dogs which he divided into three groups The first group was treated with an operation by which the nasofrontal duct was enlarged The remaining part of the frontal sinus was left untouched The dogs of the second group underwent an operation in which the mucosa of the frontal sinus was removed and the nasofrontal duct enlarged The operation performed on the dogs of the third group consisted of removal of the mucosa of the frontal sinus while the nasofrontal duct was left untouched The experimental animals were sacrificed after some time and Walsh found that the frontal sinuses of the animals of the first and second groups were both macroscopically and microscopically infected whereas those of the animals of the third group were healthy The mucosa of the frontal sinus was also completely regenerated He concluded that these experiments provided a natural explanation for the poor results of surgery aimed at enlarging the nasofrontal duct in man Surgery of the nasofrontal duct often leads to stenosis of the duct All repeated probing of the frontal sinus should also be abandoned for this reason If the nasofrontal duct is patent probing is at the least unnecessary if it is narrow the mucosa of the duct will be damaged since the duct is situated in a bony canal The results reported by Walsh show that the good clinical effect of Beck s (1916) trephination is also justified theoretically

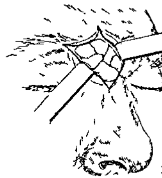
Recent frontal sinus surgery has been seeking for new ways Rhinologists well remember the good results obtained by Riedel s method of operation with regard to the infection although the cosmetic result was poor Their goal has again become the complete elimination of the frontal sinus with good cosmetic results Some studies had already been carried out and these could now be utilized Boenninghaus (1923) mentioned in his book that both Brieger and Schonborn as early as 1894 had opened the frontal sinuses by a method in which they formed from the anterior wall of the frontal sinus a lid of periosteum This remained attached to the subcutaneous tissues and was placed in position at the end of the operation The anterior wall of the frontal sinus was soon re ossified The cosmetic result was good but as regards infection the results were not usually satisfactory The mucosa of the frontal sinus used to be removed by various curettes and forceps Complete removal of the mucous membrane was not always successful with these instruments The modern magnification technique and the electric drill with cutters of various sizes and shapes, have made the complete removal of the mucosa possible Usually nowadays, after the removal of the mucosa the nasofrontal duct has been closed and the sinus filled with various tissues or filling materials Tato (1942) used fat tissue for the purpose as did Bergara & Itoiz (1955) an[d] Lemoyne et al (1968) while Schwartzman Silva (1970) used homo-grafts of preser[ved] bone Naumann (1961) plasma fibrin or [g] foam preparations Surala (1967) has [u] Ossar® a protein free preparation of cattl[e] bone Various kinds of incisions have also used Mygind (1938) and Pastore & William[s] (1938) used an incision running from one templ[e] along the hairline to the other temple T[his] incision was later used by Macbeth (19[..] Gibson & Walker (1951) Montgomery (1965) Alford et al (1965) and Aubry et al (196[.]) Others have used a skin incision running



1



2



3

Fig 1 The incision.
Fig 2 The anterior wall of the frontal sinus drilled off

Fig 3 The mosaic plastics of cortical bone

the ridge of the nose under the eyebrows (Bergara & Bergara 1935 Bergara 1947 Bergara & Itoiz 1955 Tato et al 1954 Goodale & Montgomery 1961 1964 Sirala 1967)

MATERIAL AND METHODS

The frontal sinus was first opened according to Beck A bacterial sample of the pus was taken for bacterial cultures A plastic tube was introduced into the cavity Lavage of the frontal sinus with an antibiotic solution was carried out daily through the plastic tube After the sinus infection had terminated generally after 10 days the osteoplastic operation was carried out

First a curved incision is made from the frontal process of the maxillary bone along the underside of the eyebrows The incision cuts through the periosteum but care is taken not to damage the branches of the supraorbital nerve To avoid cutting off branches of the supraorbital nerve it is important that the incision on the underside of the eyebrow should not be too long laterally For this reason a second incision is made as a rising curve forwards the forehead (Fig 1) The periosteum s detached from the bone surface Sinus fron alis is opened with an electrically driven drill nough bone to warrant good visibility into all iches of the frontal sinus is removed from the nterior wall of the sinus by drilling (Fig 2) he next step is to remove all the mucosa using operating microscope Different excavators and rosedrill cutters of various sizes are used for the purpose The mucosa of the upper part of the nasofrontal duct is removed carefully Some bone tissue both corticalis and spongiosa is now taken from the iliac crest. A bone peg with one longitudinal half of corticalis and the other of spongiosa is first formed The peg is pushed tightly from above into the nasofrontal duct It fits in tightly because the spongiosa assumes the shape of the duct. The frontal sinus is then filled with spongiosa After the whole frontal sinus has been filled with spongiosa a mosaic of cortical bone is built to fit closely into the large aperture in the anterior wall of the frontal sinus (Fig 3) In this way it is easy to build up the whole anterior wall The wound is closed layer by layer No drain is used The wound is dressed with a compression bandage

If both frontal sinuses are affected a second incision is made on the contralateral side after which the operation proceeds exactly as described The operations were performed under intubation anesthesia

The total number of patients operated was 11 6 women and 5 men Their ages ranged from 27 to 70 years The series consisted of cases in which medical therapy and other surgery (polypectomy resection of the ethmoidal cells correction of deviated or deformed septum operation of the maxillary sinuses Beck s trephination) had failed We gave up all attempts to probe the nasofrontal duct since we

Kilian s method had proved a failure the rhinologists began to devote attention to the opening and enlargement of the nasofrontal duct Halle (1915) performed a transnasal operation which however proved to be technically difficult and even dangerous (Boenninghaus 1928, Marx, 1949) The extranasal route to the frontal sinus proved to be less dangerous and there are several methods of operation based on this principle (Jansen 1894 Ritter 1906 Barany, 1924 Seiffert 1947, Uffenorde 1952) Relapses after these operations were also numerous Hajek wrote in 1936 that during his 45 years of work the treatment of chronic frontal sinusitis had given him much trouble and work but little satisfaction Walsh (1943) tried to discover why the results were poor He experimented on dogs, which he divided into three groups The first group was treated with an operation by which the nasofrontal duct was enlarged The remaining part of the frontal sinus was left untouched The dogs of the second group underwent an operation in which the mucosa of the frontal sinus was removed and the nasofrontal duct enlarged The operation performed on the dogs of the third group consisted of removal of the mucosa of the frontal sinus while the nasofrontal duct was left untouched The experimental animals were sacrificed after some time and Walsh found that the frontal sinuses of the animals *of the first and second groups were both macro*scopically and microscopically infected whereas those of the animals of the third group were healthy The mucosa of the frontal sinus was also completely regenerated He concluded that these experiments provided a natural explanation for the poor results of surgery aimed at enlarging the nasofrontal duct in man Surgery of the nasofrontal duct often leads to stenosis of the duct All repeated probing of the frontal sinus should also be abandoned for this reason. If the nasofrontal duct is patent probing is at the least unnecessary if it is narrow the mucosa of the duct will be damaged since the duct is situated in a bony canal The results reported by Walsh show that the good clinical effect of Beck s (1916) trephination is als justified theoretically

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MANDIBULAR AMELOBLASTOMA WITH PULMONARY AND MEDIASTINAL LYMPH NODE METASTASES

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Abstract A 41 year-old man with ameloblastoma of the mandible was treated with surgical resection in 1959 In 1969 a metastatic lesion was found in the lung and mediastinal lymph node metastases were diagnosed The metastatic route is discussed In the very few cases hitherto reported the interval between histologic diagnosis and proved lung metastases has been very long

The ameloblastoma comprises about 1% of tumours and cysts in the jaws (Gorlin et al 1961)

The average age of patients with ameloblastoma at onset of symptoms has been re ported to be 30–40 years (Gorlin et al 1961 Small & Waldron 1955)

More than 80% of these tumours occur in the mandible with the molar ramus region the most frequent site of involvement They are rather radioinsensitive and have a high recurrence rate Treatment should therefore be ex cision with a wide margin The roentgenogra phic appearance of ameloblastoma is normally described as a multilocular radiolucency but it can be unilocular and may be difficult to dif ferentiate from other tumours and cysts in the jaw Therefore the diagnosis of ameloblastoma should be made by biopsy and not only by roentgenography (Small & Waldron 1955)

The ameloblastoma is usually considered to be a benign tumour which normally does not metastasize but it has also been called a locally malignant tumour (Gorlin et al 1961 Small & Waldron 1955) However the literature survey and case report published by Harrer & Pat chefsky noted 8 previous cases where the tumour seems to have metastasized (Simmons 1928 Vorzimer & Perla 1932 Schweitzer & Barnfield 1943 Lee et al 1959 Tsukada et al 1965 Hohe & Harrelson 1967 Lash & McCoy 1969 Harrer & Patchefsky 1970) The purpose of this report is to add one more well documented closely followed case to the literature

CASE REPORT

In 1958 during the course of routine dental examination of the patient a 41 year old male a painless enlargement of the mandible was discovered The roentgenogram showed a mc nonuclear bone defect An ameloblastoma [illegible] residual cyst was suspected A dental [illegible] performed a local excision and removed solid tumour that was shown by [illegible] examination to be an ameloblastoma The pa tient was immediately admitted to Sjukhuset where a partial resection was formed

In 1959 a hard enlargement again was in the operated area and a biopsy show ameloblastoma An osteotomy through the veolus of the extracted left lower lateral incis was performed and another osteotomy at angulus The left corpus mandibulae was [illegible]



Fig 1 (*a*) Chest X ray in November 1969 Tumour like infiltration in the apical segment of the right upper lobe (*b*) In October 1970 confluence of the pulmonary and mediastinal infiltrations and atelectasis of the apical segment of the right upper lobe i found radiologically

sected The central part of it was totally destroyed by a white greyish homogenous tumour mass The pathologic examination showed ameloblastoma with a margin free of tumour Since 1959 the oral condition of the patient has been monitored annually both clinically and radiographically

In September 1969 the patient became troubled by an irritant dry cough A chest radiograph about a month later revealed a tumour like infiltrate 2 1/ cm in diameter situated in the apical segment of the right upper lobe The patient was admitted to the Medical Thoracic Clinic A transthoracic needle biopsy showed malignant cells of uncertain origin In order to investigate operability mediastinoscopy was performed It showed in the angle between the trachea and the right main bronchus a large packet of hard grey partly necrotized lymph nodes Histological examination showed lymph node metastases from the previously diagnosed ameloblastoma Because of the mediastinal metastases the patient was considered inoperable and left the hospital with just symptomatic therapy for his cough A chest X ray in March 1970 showed some progress of the pulmonary tumour and an infiltration in the upper part of the right pulmonary hilus and in the mediastinum In July 1970 it could be seen that the infiltrations in the right upper lobe and in the mediastinum had become confluent An X ray in October 1970 showed some further progress The patient at that time still had an irritant dry cough but remained in a good general condition Routine hematological tests and sedimentation rate were normal He was still occupationally active

PATHOLOGY

In the biopsy material removed from the mandible in March and April 1958 the microscopic picture was typical of an ameloblastoma (Fig 2) In both specimens the tumour was characterized by odontogenic epithelium arranged in cords and sheets Columnar cells were seen especially in the periphery of the epithelial islands of cells In the central epithelial areas a loose network of cells was seen sometimes resembling squamous cell epithelium Small portions of the tumour seemed to be less differentiated with irregular masses and strands of atypical epithelial cells some with mitotic figures In the specimen

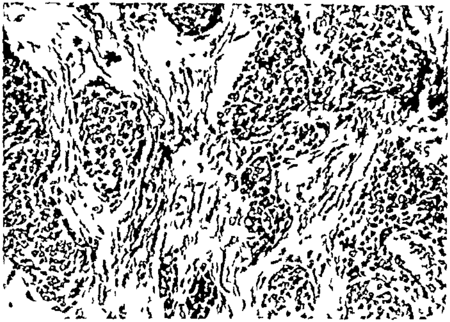


Fig 2 Histological appearance of the biopsy material surgically removed from the mandible in 1958 The ameloblastoma is characterized by a loose network of odontogenic epithelial cells in part surrounded by columnal cells Haematoxylin-eosin ×200

at the operation for the local recurrences 9 months later the same histological picture was observed

Ten years later when the patient appeared with radiological and cytological evidence of a tumour in the lung a mediastinoscopy was performed At this time a few enlarged lymph nodes were removed The lymph nodes were firm and white Histologically they demonstrated metastatic growth of a fairly well dif

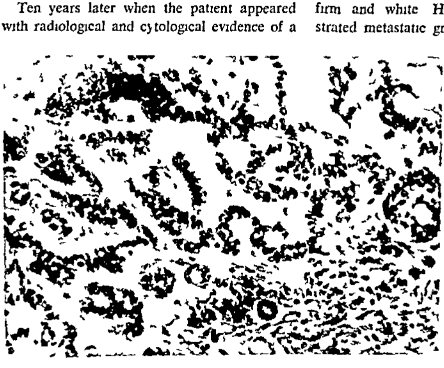


Fig 3 Lymph node metastasis of ameloblastoma In this area the tumour was of plexiform type Haematoxylin eosin ×200

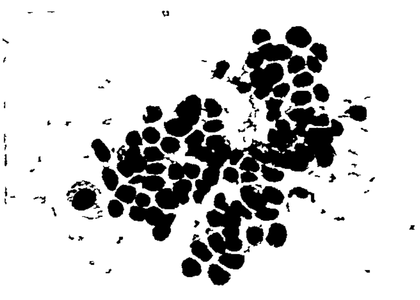


Fig 4 Cytolo_ɔical findings in aspirated cell material A cluster of fairly monomorphous tumour cells is seen Haematoxylin-eosin × 505

erentiated follicular ameloblastoma of the ame type as seen in the tumour removed from eft mandible 10 years earlier In the lymph ode metastases the tumour even demonstrated plexiform pattern (Fig 3) In small areas he lymph nodes were necrotic or fibrous It vas not possible with the biopsy material avail ble to demonstrate any invasion of the lymph essel

CYTOLOGY

n November 1969 a transthoracic needle iopsy was performed in order to obtain a orphological diagnosis of the radiologically etected lesion in the right lung At the first uncture cells were aspirated which were as ımed cytologically to represent a small cell naplastic carcinoma As the picture was not pical for a bronchial carcinoma a second ne needle aspiration was performed The cel lar material obtained demonstrated the same tological findings i e a small cell anaplastic ɪrcinoma Some cells appeared to be of epi rmoid type (Fig 4)

The cytological cell findings were then mpared with the histological picture of the ameloblastoma surgically removed from the left mandible As far as could be determined the cell structures appeared to be identical in the two specimens The lesion in the right lung was in that way diagnosed as metastasis from the ameloblastoma This diagnosis was further confirmed by the histological findings in the lymph nodes removed from the mediastinum

DISCUSSION

All the previously reported cases of metastatic ameloblastoma had one or multiple recurrences with all but one localized to the left mandible The interval between histologic diagnosis and proven metastasis can be very long in this case almost 11 years Intervals up to more than 30 years have been reported (Tsukada et al 1965)

The metastatic route has been discussed Some of the earlier cases have been considered to be metastatic lesions caused by tumour aspiration (Vorzimer & Perla 1932 Schweitzer & Barnfield 1943 Lash & McCoy 1969) but cases with lymphatic and blood vessel te sion have also been demonstrated (

ously he had full radiation therapy (Telekobalt 6 000 R) which was not successful He developed fair esophageal speech which did not satisfy him for psychic reasons Because of his very active temperament he felt severely handicapped when discussing and debating with his customers and felt that his speech was not up to the demands of his extroversive temper Thus he retired more and more from his previous activities and became a tremendous problem to his family Thus we performed an Asai procedure on him in the winter of 1967/68 With postoperative dilatation we obtained a patent pharyngo-tracheal tube Unfortunately the functional outcome was a complete failure The patient suffered from massive aspiration of saliva and food and his voice remained a weak whisper The reason for this may have been an extraordinarily short and large tube as well as the location and configuration of the pharyngeal opening We therefore offered the patient a choice between returning to his previous unsatisfactory esophageal speech or a revision of the Asai procedure Our operative plan was to build an intra esophageal bar which would act as a *pseudo-epiglottis* just above the pharyngeal opening of the Asai tube

METHOD

Skin of the right paramedian side of the and submandibular region is used to prepare a tubed pedicle flap (Fig 1)

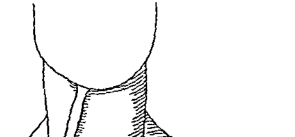


Fig 1 Pedicle flap from the healthy part of the anterior neck

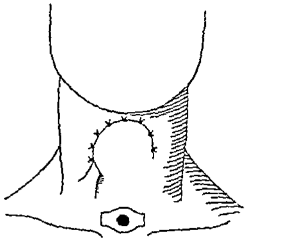


Fig 2 Submental skin flap replaced after suturing pedicle flap to esophageal wall

Six weeks later the submandibular root of the tube is detached and the submandibular wound closed A skin flap from the submental region about 5 cm large at the base is mobilized downwards to the region of the interior opening of the tracheoesophageal Asai fistula To delineate the location where the pseudo-epiglottis is to be inserted needles are placed into the anterior esophageal wall and controlled by esophagoscopy A horizontal slit is cut into the

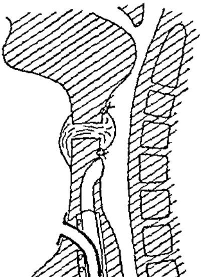
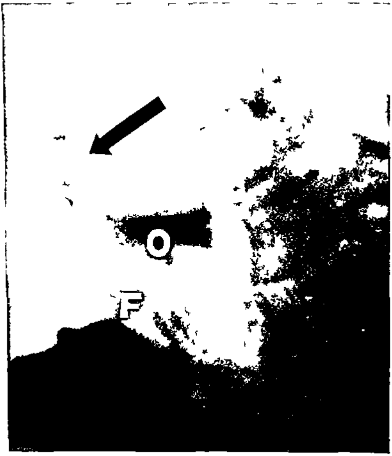


Fig 3 Lateral view of insertion Asai fistula



anterior wall of the esophagus and the submandibular end of the pedicle flap sutured to the edges of the slit in two layers (Figs 2 3) The flap bulges horizontally into the esophageal space thus forming a pseudoepiglottis just above the pharyngeal opening of the Asai fistula At the same time it narrows the lumen of the pharynx The epidermal surface of the middle part of the tube of course had to be removed before replacing and suturing the submental skin flap into its original bed (Fig 3)

RESULT (Fig 4)

After healing by primary intention the voice of the patient is perfectly understandable and intensive It has a regular flow and good modulation Speech continues to improve and especially in the eve...

cellent There is ...
but the voice ...
moderately h...
tracheal tube ...
have to use ... h
when talking ... nd
and Asai's c... iow
When foo... ion
no difficulty ... the
may couse ... n et
ing large m... heir
respiratory ... in a
tracheal tu... 966
the trache... cells
by salivary ... more
pletely dr...
Accord...

life has become again enjoyable for him His psychic problems and his family problems have been completely overcome

DISCUSSION

The problem of surgical rehabilitation of the laryngectomee has been revived by Asai s publication of 1965 Yet we must not overlook the fact that the outcome of this kind of intervention may be hazardous Stenosis location size of the Asai tube and its openings in the pharynx and trachea may produce an unfavorable result Speech production is frequently no special problem in these cases however appropriate closure of the airway during deglutation remains the crucial problem Ways must be found for plastic reconstruction of a mobile pseudo-epiglottis to fulfil all functional requirements The tubed pedicle skin flap as described above is one promising solution Special attention must be given to the size and the placement of the intraesophageal insertion of the flap McGrail with his one stage rehabilitation by a chest flap offers another very promising technique but this author also has problems with aspiration

RÉSUMÉ

s presentons une technique operatoire qui ame ore le resultat d une intervention selon ASAI Un lambeau cutane sert de Pseudoepiglotte—pseudoglotte qui permet la formation d'une voix sonore satisfaisante et empeche l aspiration de la salive

ZUSAMMENFASSUNG

Weiterentwicklung der ASAI schen Operationstechnik zur sprachlichen Rehabilitierung Laryngektomierter Mit einem gestielten Hautlappen wird uber dem Eingang der Asai'schen Fistel eine Pseudoepiglottis gebildet, die das Oesophaguslumen einengt und neben der Behinderung der Speichelaspiration auch zur besseren Stimmbildung beitragt

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THE SENSORY HAIRS AND THE TECTORIAL MEMBRANE IN THE DEVELOPMENT OF THE CAT'S ORGAN OF CORTI

A Scanning Electron Microscopic Study

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Abstract The surface topography of the organ of Corti has been studied by scanning electron microscopy Newborn kittens of various ages as well as adult cats were included in this study In the newborn kitten all the sensory cells have one kinocilium in addition to the bundle of stereocilia This kinocilium most frequently is short and looks rudimentary however some of the kinocilia are even longer than the longest of the stereocilia The distal end often shows a bulb like thickening Within a few weeks after birth all the kinocilia disappear The relationship between the outer margin of the tectorial membrane and the epithelial surface has been investigated In the kitten the tectorial membrane is firmly attached at its outer margin to the upper free surfaces of the outermost row of Deiters cells There is no such attachment in the cat six weeks of age nor in the adult cat This may have functional implications

Stimulation of the sensory cells in the cochlea is generally considered to be the result of a shearing motion between the tectorial membrane and the organ of Corti We believe that the stimulus acts primarily on the hairs of the sensory cells and the excitation thus aroused is subsequently transmitted to the central nervous system in re coded form

The mechanoreceptors in the inner ear are all provided with hairs or cilia In the vestibular sensory regions these are of two types each sensory cell being equipped with one kinocilium and several stereocilia Observations by light microscopy (van der Stricht 1908 Held 1909 1926 Kolmer 1927) indicated a similar differentiation of the hairs in the mammalian organ of Corti however recent electron microscopic studies have clearly shown that whereas a kinocilium is present during fetal and very early post natal life (Kikuchi & Hilding 1965 *a b* Kimura 1966 Wersall & Flock 1967) the sensory cells in the mature organ of Corti have no kinocilium On the other hand the basal body from which the kinocilium originally protruded is found in the adult cochlea It is situated just below the free surface of the sensory cell in a region devoid of cuticular material external to the associated bundle of stereocilia (Engstrom et al 1966) The stereocilia are attached by their rootlets to the cuticle of the sensory cells in a constant pattern (Held 1926 Kimura 1966 Wersall & Flock 1967) On the outer hair cells the stereocilia are arranged in three or more rows forming a W pattern This W form is less visible on the inner hair cells where the

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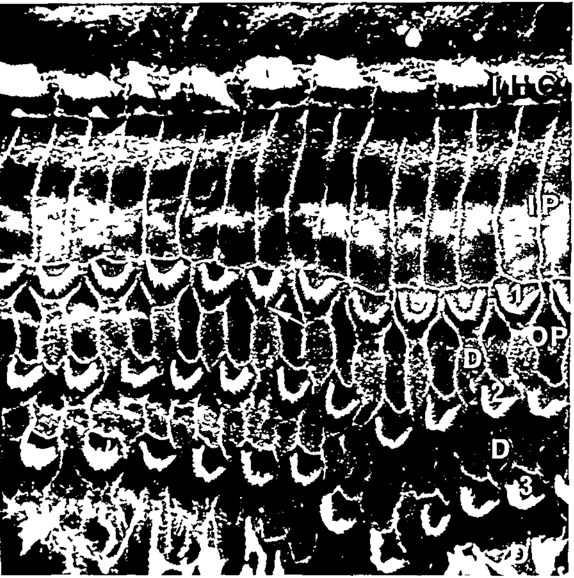


Fig 1 Outer hair cells from the apical coil of the cochlea in the two day old kitten Each sensory cell equipped with one kinocilium (*arrow*) located ex nal to the bundle of stereocilia (*S*) The kinocilium ionally appears to extend from a hillock (*) which is situated nearer to the stria vascularis than is the kinocilium This hillock probably represents an artefact The Deiters cells in the newborn cat are provided with long microvilli (*m*) Magnification × 7 900

cilia are arranged in almost straight rows paral lel to the spiral duct Studies by light micro scopy (Held 1909 1926 Kolmer 1927) showed variations in the length of the stereocilia These observations were recently extended by Ki mura (1966) with the aid of electron micro scopy On each cell the longest hairs are found in the outermost row the length of the hairs in the inner rows decreasing stepwise Moreover the hairs of the cells in the apical turn of the cochlea are taller than those of the cells in the basal turn (for further information on the sensory hairs see Kimura 1966)

The jelly like tectorial membrane extends from the spiral limbus and covers the organ of Corti The relationship between its outer mar gin and the epithelial surface has been a matter of controversy It is still not known whether or not the tectorial membrane is attached to the epithelial surface outside the hair cell area

The purpose of the present study was to fol low the development of the sensory hairs and study the relationship between the outer mar gin of the tectorial membrane and the epi thelial surface in kittens of different ages The



Fi 2 O gan of Corti from the middle coil of a 16 day old kitten The sensory cells are arranged in one row of inner hair cells (*IHC*) and three rows of outer hair cells (1 2 3) The sensory cells are enclosed in a framework of supporting cells the inner (*IP*) and th out r (*OP*) pillar cells and the Deiters cell (*D*) Only one kinocilium (*arrow*) is visible The appearance of the microvilli is similar to that in the adult cat Magnification ×2 200

studies were based on scanning microscopy a technique particularly well suited to the studies of surface structures

MATERIAL AND METHODS

The temporal bones of nine kittens aged from two days to six weeks were used in these studies as were those from several adult cats The animals were decapitated under Nembutal anesthetic (intra peritoneal) and the temporal bones removed and the bullae opened The round window membrane was perforated the stapes foot plate detached and the inner ear perfused with cold 1 or 2 osmic acid solu tion in veronal or phosphate buffer The temporal bones were kept in the fixative for two hours at about 4 C After rinsing

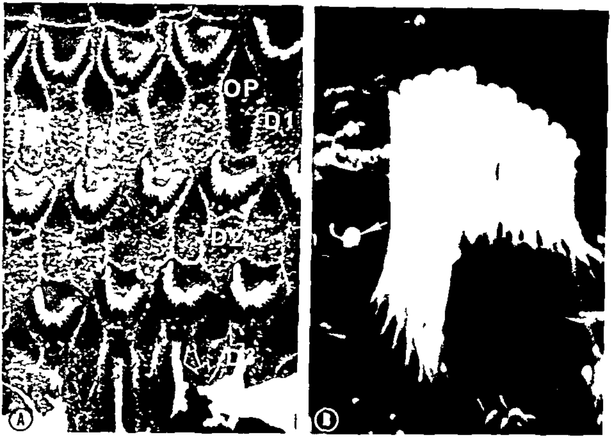


Fig 3 (A) Outer hair cells from the middle coil of a 16 day old kitten The stereocilia on each cell form a W pattern The microvilli are more numerous on the Deiters cells (D 1 D 2 D 3) than on the outer pillar cells (*OP*) Remnants of the marginal part of the tectorial membrane (*open arrow*) is attached to the upper surface of the outermost Deiters cells Magnification × 2 600

(B) Hair bundle from the apical coil of 16 day old kitten The stereocilia vary in length the outermost being the longest and the innermost the shortest Arrow indicates rudimentary kinocilium Magnification × 15 300

Ringer solution one ear was dissected for light microscopy using the surface specimen technique (Engstrom et al 1966) The other ear was prepared for scanning electron microscopy (Bredberg et al 1970) In some cases specimens used for light microscopy were subsequently prepared for scanning microscopy The light microscopy was carried out with a Nikon phase contrast microscope the scanning microscopy with a Cambridge Stereoscan scanning electron microscope

OBSERVATIONS

The surface topography of the organ of Corti in the two day old kitten differs in several re pects from that of the adult cat The organ of Corti appears narrower in light microscopy giving the illusion that the sensory cells are more densely packed than in the adult animal This agrees with observations on the fetal and adult human cochlea reported by Bredberg (1968) Scanning electron microscopy under higher magnification reveals a striking difference between the newborn kitten and the adult cat in the appearance of the microvilli on the Deiters cells In the two day old animal (Fig 1) the microvilli particularly in the apical part of the cochlea are several times longer than those of the adults however by the end of a week a substantial reduction in the length of the microvilli has occurred (Fig



Fig 4 Inner hair cell from the middle coil of kitten 16 days old as seen from modiolus The gradation in the length of the stereocilia is demonstrated Not very long and thin kinocilium with a bulb-like distal thickening (*arrow*) Magnification × 16 900

9 A) and sixteen days after birth the appearance of the microvilli does not differ significantly from that in the adult cat (Fig 2) The microvilli are more numerous on the Deiters cells than on the pillar cells (Figs 2 3 A)

In the newborn animal all of the hair cells of the organ of Corti are provided with kinocilia situated just external to the W shaped bundle of stereocilia (Fig 1) In most cases the kinocilia look rudimentary being shorter than the longest of the stereocilia The kinocilium occasionally appears to extend from a hillock however this hillock which is actually a protrusion of cytoplasm into the endolymphatic space is situated farther out than is the kinocilium It may be a post mortem extrusion of cytoplasm at the point where the cuticle of the upper free surface of the sensory cell is absent a similar phenomenon being seen on vestibular sensory cells

By the end of the first week of postnatal life the sensory cells have begun to lose their kinocilia They have almost entirely disappeared from the middle portion of the organ of Corti while in the apical and basal coils they are still intact During the second week most of the remaining kinocilia disappear In Fig 2 which shows a segment of the lower apical coil in a 16 day old kitten a kinocilium is observed only on one of the outer hair cells Figs 4 and 5 A from another kitten of the

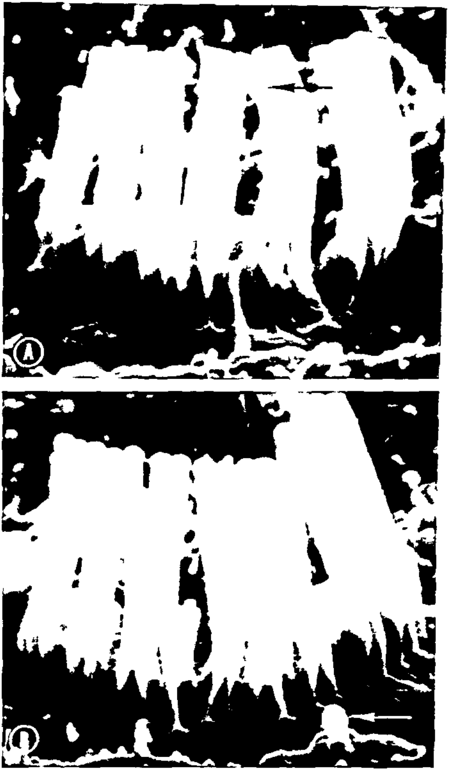


Fig. 5 (A) Inner hair cell from the same area as in Fig. 4 as seen from the stria vascularis. Arrow shows bulb-like thickening of the distal end of the kinocilium. Magnification ×16 200.

(B) On a neighboring inner hair cell the kinocilium has almost completely disappeared however a *(arrow) indicates the rudimentary* kinocilium the narrow basal part of the stereocilia, attaching hairs to the cuticular plate of the cell. Magnification 17 500

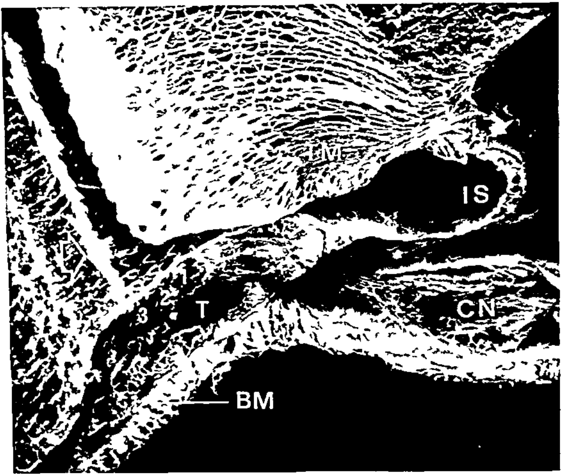


Fig 6 Fracture through the middle coil of the organ of Corti in the one week old kitten The hair bundles of the outer hair cells (1 2 3) are seen The tectorial membrane (*TM*) extends from the spiral limbus (*SL*) and covers the organ of Corti It is attached at its outer margin to the outermost Deiters cells (*arrow*) however during the preparation procedures it has been broken off at some distance from the epithelial surface *CN* = cochlear nerve *BM* = basilar membrane *T* = tunnel of Corti *IS* = inner sulcus Magnification ×750

same age demonstrate under higher magnification two inner hair cells where the kinocilia are still intact These kinocilia appear to be longer than the longest of the stereocilia and even thinner except for the most distal part which shows a bulb-like thickening They also seem to be more flexible than the stereocilia A neighboring sensory cell (Fig 5 B) like most of the hair cells at this stage has almost lost its kinocilium only a short knob protruding from the epithelium to mark its passing Later even this knob disappears In the three and one half week old kitten very few kinocilia were observed and in the six week old animal none were seen

In the scanning microscope the form of the hair bundles as well as the individual stereocilia is clearly demonstrated confirming earlier observations by transmission electron microscopy (Kimura 1966) The gradation in the length (Figs 3 B 4) of the stereocilia (the innermost row being the shortest and the outermost row the tallest) is more pronounced in the apical part of the cochlea than in the basal As can be seen in Fig 1 the stereocilia in the outermost row are longest close to the



Fig 7 Organ of Corti with tectorial membrane (*TM*) in the apical coil of kitten 16 days old At the extreme left the tectorial membrane is attached to the epithelial surface at its outer margin however most of the tectorial membrane has detached marginally thus permitting view of the hair bundles of the sensory cells *IHC* = inner hair cells *I* = first row of outer hair cells Arrows indicate remnants of the marginal part of the tectorial membrane on the outermost Deiters cells *Magnification* ×*320*

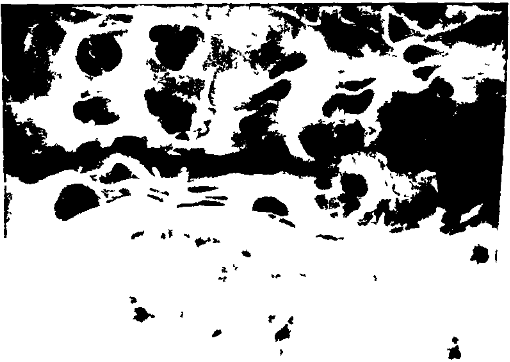
base of the W The length of the longest stereocilia gradually diminish from the apical l toward the basal coil The form of the idual stereocilia can be seen in Figs 3 B 5 A B which show the attachment of the stereocilia by their very narrow basal portion to the cuticular plate of the sensory cell The individual hairs of the outer hair cells are thinner than those of the inner hair cells and appear to be more densely packed on the cuticular plate

The tectorial membrane covers the hair cell area completely The tectorial membrane shows a fibrillar pattern the fibrils being arranged mainly in a radial and slightly apical direction as previously described (Siebenmann 1899 Kolmer 1927 de Vries 1949 and others) In the kitten the outer margin of the tectorial membrane is attached to the epithelial surface just outside the third row of outer hair cells (Figs 6 7 8 A B) This marginal attachment of the tectorial membrane varies in the different parts of the cochlea In the apical coil an attachment by rather long irregularly distributed extensions of the tectorial membrane is seen These extensions form a sort of marginal network which is attached to the outermost Deiters cells and close to the apex possibly make contact with some Hensen cells also In the lower apical coil and from there toward the base the marginal attachment of the tectorial membrane becomes

Fig 8 (A) Middle coil of kitten two days old To the right side of the arrow the tectorial membrane is attached to the Deiters cells at its outer margin Magnification ×390

(B) This shows by higher magnification the trabecular extensions (*open arrows*) of the tectorial membrane attached to the outermost row of Deiters cells Solid arrow points to kinocilium on a hair cell Magnification ×3 400


TM



more and more regular due to the regular arrangement of the outermost Deiters cells This can be seen in Fig 8 A B from the upper basal coil showing short trabecular extensions of the tectorial membrane attached to the free surface of the outermost row of Deiters cells the number of attachments corresponding fairly well to the number of cells in the third row of outer hair cells (Fig 9 A B C) During the dissection chemical fixation or freeze drying procedure the tectorial membrane often detaches marginally and can be seen to be displaced from the organ of Corti In these cases however it is always detached at some distance from the epithelial surface leaving a column like remnant on each of the Deiters cells (Figs 3 A 6 7 9 A B C) In the four week old kitten the marginal attachment is visible However in the six week old kitten as well as in the adult cat no sign of such attachment is seen

In both the kitten and the adult cat additional areas of contact between the epithelial surface and the underside of the tectorial membrane were scrutinized In particular the area medial to the inner hair cells just opposite to the Hensen stripe of the tectorial membrane was carefully inspected Even in the newborn kitten remnants indicating an attachment of the tectorial membrane to this area were never seen

DISCUSSION

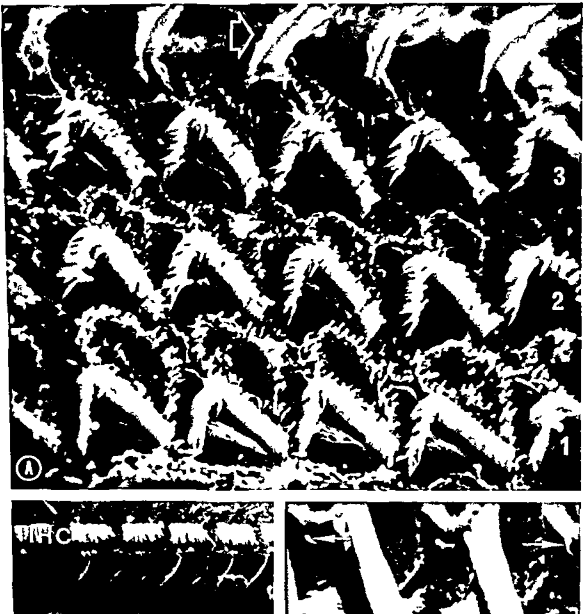
The relationship between the tectorial membrane and the organ of Corti has been a matter of much dispute The main reason for this is undoubtedly the technical difficulty in preserving the normal form and position of the tectorial membrane in histological studies The tectorial membrane like the cupula in the vestibular labyrinth is highly vulnerable to the fixation and embedding procedures which alter both its shape and position above the organ of Corti Thus conclusions from histological studies concerning its form and attachments must be drawn very carefully In light microscopy the tectorial membrane is usually seen floating free above the organ of Corti attached to the spiral limbus but otherwise in contact with neither the sensory hairs nor with other areas of the epithelium This certainly does not represent its normal position since several studies have shown that in mammals at least the longest of the hairs of the outer hair cells are in contact with the lower surface of the tectorial membrane (Iurato 1961 Kimura 1966 Bredberg 1968 Hawkins & Johnsson 1968 Spoendlin 1968)

There seems to be general agreement that during fetal life the tectorial membrane has a closer relationship to the underlying epithelium than in adults a fact Held (1926) explains on the basis of the embryological development Kolmer (1927) held the opinion that during the entire fetal life the tectorial membrane is attached by fine threads to the upper surface of the supporting cells surrounding the sensory cells This arrangement is also found in the mature organ of Corti of birds and reptiles (Held 1926 Kolmer 1927 Jahnke et al 1969) but has not been demonstrated in adult mammals

Fig 9 (A) Outer hair cells (*1 2 3*) in the basal coil of the cochlea in the one week old kitten as seen from the modiolus The stereocilia are clearly shorter than they are in the apical coil Note the rather long microvilli on the Deiters cells The number of marginal attachments of the tectorial membrane corresponds to the number of Deiters cells (and also to the number of cells in the third row of hair cells) Magnification ×6 200

(B) Basal coil from the 16 day old kitten Due to a fracture of the organ of Corti just external to the hair cell area the third row of hair cells (*3*) and the phalangeal processes of the outermost row of Deiters cells (*D*) are seen The free surface of the outermost Deiters cells are covered with remnants (*arrow*) of the marginal extensions of the tectorial membrane *IHC* = inner hair cells Magnification = ×1 900

(C) In the basal coil of the two day old kitten the marginal extensions (*open arrow*) of the tectorial membrane are seen Each sensory cell is provided with a kinocilium (*small arrows*) Magnification ×6 900


3
2
1
A
B
D
D
3
3
C

The question remains as to whether or not the tectorial membrane normally makes contact with the underlying epithelium at its outer margin Electron microscopic studies (Kikuchi & Hilding 1965 *a* Kimura 1966) showed that the tectorial membrane in the newborn mouse was attached at its margin to the epithelial surface thus confirming earlier observations by light microscopy (Held 1909 1926 Kolmer 1927) however in specimens from adult animals no attachments were ob served (Kimura 1966) We arrived at the same conclusion in the present study observing a marginal attachment in the kitten up to the age of four weeks but not in a kitten of six weeks nor in the adult cat The attachment was discontinuous consisting of trabecular extensions of the tectorial membrane to the surface of the epithelium During the prepara tion procedure the tectorial membrane was frequently broken off near its outer margin however in these cases it was broken off at some distance above the epithelial surface leaving a column of material on each of the Deiters cells Thus the tectorial membrane was *never* detached *at* the epithelial surface It seems justified therefore to conclude that in he newborn kitten the tectorial membrane rather firmly attached to the epithelial sur-

Using different techniques and avoiding chemical fixation it has been suggested that the tectorial membrane is attached marginally also in the mature organ of Corti De Vries (1949) suggested that any detachment of the tectorial membrane was due to the preparation procedures although he only occasionally ob served a marginal attachment Hilding (1952) basing his studies mainly on human material concluded that the outer margin of the tecto rial membrane which made contact with the epithelial surface extended to the base of the Hensen cells Tonndorf et al (1962) using in travital stains found the tectorial membrane so firmly attached to the Hensen cells by means of its outer marginal area that the stains did not enter below it Based on the foregoing conclusions Rauch (1964) con sidered that the tectorial membrane the inter nal spiral sulcus and the reticular membrane formed a closed space possibly filled with a special fluid

The present study does not support Rauch s interpretation Even in the newborn kitten the marginal attachment was seen to be non continuous apparently permitting open com munication between the endolymphatic and subtectorial space Therefore it seems un likely that there should exist any closed sub tectorial space filled with a special lymph in the mature organ of Corti however since the present study was based entirely on fixed specimens it does not allow definite conclu sions on the issue On the other hand the study does permit the following conclusion In the kitten the tectorial membrane is rather firmly attached by its marginal network If there is any marginal attachment in the adult cat it is certainly not as firm as in the kitten Fur thermore the marginal extensions of the tec torial membrane do not makes contact with the Hensen cells as often described but with the third row of Deiters cells

From a functional point of view it appears likely that the hairs of the inner hair cells are in contact with the tectorial membrane but such a contact has not yet been verified histologically It has also been suggested that an attachment of the tectorial membrane to the underlying epithelium exists at the stripe of Hensen (Hilding 1952 Lim & Lane 1969) In the present study the epithelial surface medial to the inner hair cells was inspected for gelatinous material which would indicate an attachment of the tectorial membrane at this point but it was not seen

The difference in the surface topography between the newborn animals and the adults is interesting not least from a functional point of view since it is known that in the newborn kitten as well as in other newborn animals hearing is not yet developed The present study confirms in general previous observations by light and electron microscopy In the kit

ten Kikuchi & Hilding (1965 *a*) observed an abundance of long (1–3 μ) microvilli on the supporting cells by transmission electron microscopy whereas in the adult the micro villi were shorter (0 25 μ) and less numerous The present study showed that in the one week old kitten there was already a clear cut reduction in the size of the microvilli which at 16 days appeared of normal adult size They were numerous on the Deiters cells in contrast to the outer pillar cells which were almost devoid of microvilli The long microvilli seen in the newborn and their close relationship to the tectorial membrane during fetal life (Kol mer 1927) may indicate a role in the forma tion of the tectorial membrane Attempts have been made to correlate the structural development of the organ of Corti with auditory func tion (Larsell et al 1944 Alford & Ruben 1963 Kikuchi & Hilding 1965 *a* Mikaelian & Ruben 1965) In the mouse auditory function becomes evident by the 9th to the 14th day (Al ford & Ruben 1963) and the cochlear poten tials attain normal adult values by the 14th day at which time the organ of Corti is structurally mature (Mikaelian & Ruben 1965) Thus the structural development of the organ of Corti in the mouse correlates well with the results of behavioral and electro physiological studies

In the present study the surface topography of the organ of Corti of the cat appeared mature two to three weeks after birth As judged by scanning microscopy the marginal detachment of the tectorial membrane was the last event in the maturation of the cochlea of the cat It is tempting to speculate whether this detachment may be of importance for auditory function since Fernandez (personal commu nication) found that the cochlear microphonics in the cat attained their normal adult values four to five weeks after birth

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ZUSAMMENFASSUNG

Die Oberflachenstruktur des Cortischen Organes ist mit „Scanning Electron Microscopy" studiert worden Die Studie wurde an neu eborenen sehr jungen und vollausgewachsenen Katzen vorg nommen Bei den neugeborenen Katzen haben samtliche Sinneszellen ein Bundel von Stereozilien Das Kinozilium ist ge wohnlich kurz und sieht rudimentar aus jedoch können einige Kinozilien auch langer als die langsten Stereozilien sein Die distalen Enden zeigen oft eine Kolbenahnliche Verdickung Innerhalb einiger Wochen nach der Geburt verschwinden sämtliche Kinozilien Das Verhaltnis zwischen dem ausseren Rand der Tectorialmembran und der epithelialen Oberflache des Cortischen Organes wurde besonders studiert Bei den jungen Katzen ist der aussere Rand der Tectorialmem bran an den frei n Oberflachen der D itersz llen befestigt Weder bei den sechs Wochen alten noch bei den ausgewachsenen Katzen gibt es solche Be festigungen Die funktionelle Bedeutung wird disku tiert

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FIBER ARRANGEMENT IN THE TYMPANIC MEMBRANE

Scanning Electron Microscope Observations

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Abstract The fiber arrangement of the intermediate fibrous layer of the guinea pig tympanic membrane was observed by scanning electron microscopy The intermediate fibrous layer consists of radial fiber bundles and not radially oriented fiber bundles The latter is subdivided into four groups parabolic circular oblique fibers and the annular ligament The directional organization of those fiber bundles is demonstrated The oblique fibers consist of two distinct fiber groups termed as oblique ascending and oblique descending fibers The distribution of the non radial fiber bundles is demonstrated under three dimensional surface views and discussed concerning the vibratory pattern of the tympanic membrane

The tympanic membrane is composed of three layers an outer cutaneous an intermediate fibrous and an inner mucous The cutaneous layer derived from the skin which lines the external auditory meatus consists of stratified epithelium The mucous layer is a part of the *mucous membrane of the tympanic cavity*

The intermediate fibrous layer is the most complicated portion of the tympanic membrane This consists of two distinct fibrous arrangements circular and radial designated according to their directional trends (Politzer 1868 Gerlach 1869 von Troltsch 1858 Eggston & Wolff 1947) The fiber arrangement has been studied in relation to the mode of vibration of the membrane Fumagalli (1947) and Secondi (1951) investigated the fiber arrangement of the tympanic membrane in various animals using the polarized light microscope and reported new fiber arrangements and functional explanations for those fiber groups

The high magnification surface views with the scanning electron microscope may contribute valuable additional information about the intermediate fibrous layer to our present knowledge which is based mainly on ultrathin sections (Lim 1968 1970 Johnson et al 1968 Hentzer 1969) and polarized light examination of surface specimens (Fumagalli 1947 Filogamo 1949 Secondi 1951 Owada 1959 Kirikae 1960) Application of the scanning electron microscope seems especially suited to the examination of the fiber arrangements consisting mainly of collagen fibers because of its ability to possibility of the three-dimensional view (Hayes & Pease 1968)

MATERIAL AND METHODS

The tympanic membranes were obtained from healthy adult guinea pigs The animals were decapitated and the temporal bones removed The tympanic membrane was taken from the temporal bone under the dissecting microscope Specimens were incubated in 1% trypsin in phosphate buffer solution (Dulbecco & Vogt 1954) or 0 1 pronase at 37 C for 3 to 48 hours and then washed by a pipette with physiological saline This procedure removes most of mucous and cutaneous layers from

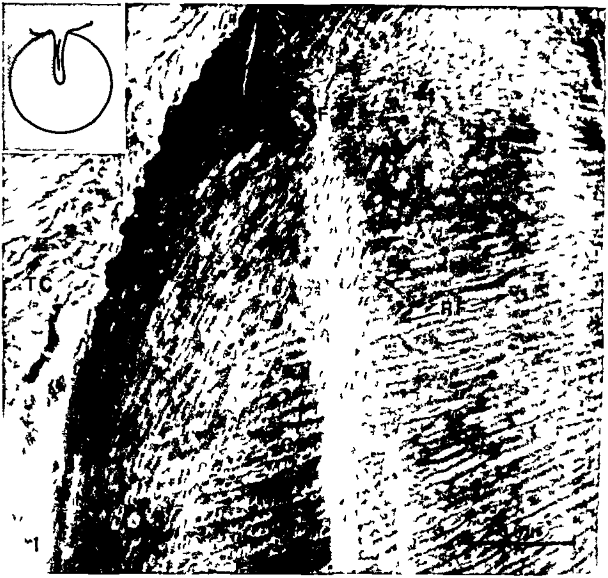


Fig 1 A low power scanning electron micrograph illustrating the fiber arrangement in the anterior superior quadrant of the tympanic membrane Radial fibers (*RF*) parabolic fibers (*PF*) circular fibers (*CF*) and the annular ligament (*AL*) run through the intermediate fibrous layer of the membrane The tympanic crest (*TC*) can be seen on the left side of the micrograph ×300

tympanic membrane leaving the intermediate fibrous layer (Johnson et al 1968) After washing in physiological saline specimens were fixed with 10% neutral formalin for 5 to 24 hours and then dehydrated with a series of ascending concentration of acetone from 50 through 70 90 and 100% Specimens were allowed to air dry and mounted on metallic specimen holders and then coated with carbon and gold on a rotating stage in a vacuum evaporator (Barber & Boyde 1968) They were examined with a JSM 3 scanning electron microscope (Japane Electron Optic Lab Co Ltd) under 10 KV accelerating potential

RESULTS

The shape of the tympanic membrane of the guinea pig is almost round with a diameter of about 6 mm The tympanic membrane is suspended at the tympanic crest which is the terminal portion of tympanic bulla within the

Table II *The accuracy at indirect recording of the middle ear pressure with three different calculation procedures*

Known middle ear pressures (cm H O)	A Recorded $Pch_{V max}$ (corr indiv) and ΔV_{tm} V_m known	B Recorded $Pch_{V max}$ (corr indiv) and ΔV_{tm} V_m assumed to be 6 ml	C Recorded $Pch_{V max}$ (corr −1 cm H_2O) and ΔV_{tm} V_m assumed to be 6 ml
+3 5	M +3 47	M +3 40	M +3 84
	S D ±0 30	S D ±0 43	S D ±0 94
	S E ±0 09	S E ±0 14	S E ±0 30
−4 0	M −3 86	M −3 80	M −3 50
	S D ±0 31	S D ±0 47	S D ±1 06
	S E ±0 10	S E ±0 15	S E ±0 34

of the middle ear of 6 ml (6 4 ml ±2 48) for an approximate calculation of the pressure change in the middle ear during pressure equilibration This assumption and the utilization of pressure change in the middle ear caused by the volume deviation of the drum increase the accuracy of the calculations as compared with the pneumophone and impedance methods This appears from the control experiments below

Control experiment

Known middle ear pressures have been produced in 5 persons with normal tympanic membranes in the following way different over and underpressures are applied to the rhinopharynx during deglutition All of them propagate these pressures quantitatively to the middle ear $Pch V_{max}$ is recorded at the applied middle ear pressures of +3 5 and −4 cm H O This is repeated twice at each pressure With the aid of $Pch V_{max}$ V_m and ΔV_{tm} the middle ear pressure has been indirectly determined in three different ways

A The middle ear pressure is calculated with the aid of recorded $Pch V_{max}$ ΔV_{tm} and V_m $Pch V_{max}$ is corrected individually The results appear from Table II and amount to −3 47 and −3 86 cm H O

B The same calculation procedure as under A with the exception that the middle ear volume is assumed to be 6 ml Mean value +3 40 and −3 80 cm H_2O The exactness of this calculation procedure will be somewhat less which is above all explained by dispersion in volumes from the assumed mean value of 6 ml

C Middle ear pressure is calculated with the aid of recorded $Pch V_{max}$ ΔV_{tm} and an assumed V_m of 6 ml $Pch V_{max}$ is corrected with the mean pressure 1 cm H O Result +3 84 and −3 50 cm H O It is clear from Table II that the accuracy decreases which is above all explained by the approximative determination of the neutral position The procedure under C is however the only available one in cases that cannot equilibrate completely To be able to record a maximum velocity of the drum the volume pressure relation of the drum must follow an S shaped curve (Fig 3)

Control experiments on these 5 subjects have also been performed at other known middle ear pressures such as +5 5 +8 5 7 and −12 cm H_2O The pressure determinations show nearly the same absolute accuracy as those accounted for above

Conclusion

The accuracy at the indirect determination of the middle ear pressure when all the variables of the middle ear are determined is ±0 6 cm H_2O If a mean volume of 6 ml is used the accuracy is somewhat less With an approximative determination of the neutral position and an assumed middle ear volume

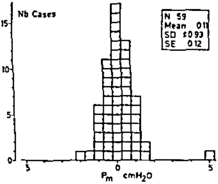


Fig 4 Indirect determination of the middle ear pressure on 59 cases with a perfect tubal function tubal function group I according to calculation procedure B

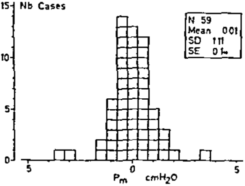


Fig 5 Indirect determination of the middle ear pressure on 59 cases with a perfect tubal function tubal function group I according to calculation procedure C

of 6 ml the accuracy decreases further (Table II) These values are obtained in subjects with normally movable drums The accuracy decreases more if the drum is flaccid

THE NORMAL MIDDLE EAR PRESSURE

Material

The material consists of 86 subjects with no present or anamnestic signs of ear disease recruited among medical students and blood donors In all cases there was a negative history of ear disease and a normal ear examination The hearing threshold was within 0–20 dB (related to ISO standard 1964) All subjects were normal at examination of the nose and throat The function of the Eustachian tube was also determined 59 subjects could equilibrate relative over and underpressures completely (group I) 20 could equilibrate to small residual pressures in the middle ear (group II) 2 were only able to equilibrate relative overpressure but not underpressure (group III) and 5 had no capacity whatsoever to equilibrate during deglutition (group IV)

Results

In the 59 subjects with good tubal function group I the normal middle ear pressure i e the pressure at the beginning of the experiment was determined indirectly according to calculation procedure B (Fig 4) To be able to compare the middle ear pressure in the whole material among which there were several cases with reduced tubal function calculation according to procedure C was also used (Figs 5 6)

Two determinations of the middle ear pressure have been performed with an interval of about 1 month in 6 cases belonging to group I and in 6 cases in groups III–IV (Fig 7)

In cases belonging to group I i e perfect tubal function the normal middle ear pressure is close to the ambient pressure (Figs 4 5) In cases with less good tubal function groups II–IV (Fig 6) the pressure is more varied It should be observed that among these cases there are seven who cannot equilibrate a relative underpressure in the middle ear by deglutition at all (groups III and IV) They must nevertheless be regarded as having normal ears The case with the high positive middle ear pressure (Fig 6) regularly uses the Valsalva manoeuvre to force air into the middle ear cavity The results in Fig 7 from cases with



Fig 6 Indirect determination of the middle ear pressure in 27 cases with a reduced tubal function, tubal function groups II–IV according to calculation procedure C

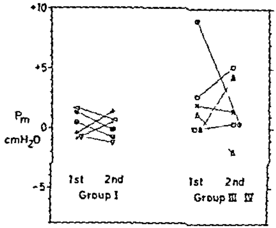


Fig 7 Repeated recording of the middle ear pressure according to calculation procedure C in 6 cases in tubal function groups I and III–IV with an interval of about 1 month

perfect tubal function show good agreement between the two recordings with 1 month s interval In groups III and IV however there is a greater variation in the middle ear pressure between the two recordings

DISCUSSION

So far the pressure balance across the tympanic membrane has generally been recorded by the pneumophone but above all by the impedance method It is therefore of great interest to compare the pressure at minimum impedance with the pressure at maximum movement velocity of the drum $P_{ch}I_{max}$ When we increase the ambient pressure from −15 to +15 cm H_2O in 25 sec we record the maximum movement velocity at an ambient pressure of −1 cm H_2O (S D ±0 92) In a study of 100 normal ears with a change of the ear canal pressure from −20 to +20 cm H_2O during 45 sec Liden et al (1970) found the minimum of impedance at −1 cm H_2O (S D ±1 50) There is thus good agreement if the recordings are made during an increase of the ambient pressure During a decrease from +15 to −15 cm H_2O we record the maximum movement velocity at −2 60 cm H_2O (S D ±1 23) Liden however did not find any difference whether the pressure was decreased or increased The maximum movement velocity of the drum is recorded at different ambient pressures at increase and decrease which is explained by the hysteresis obtained An analysis of this phenomenon is made in *A method for studies of the middle ear mechanics* by Elner et al (1971)

By the present method valuable investigations *can be performed if the subjects can equilibrate* completely and the middle ear variables can be determined We can follow the middle ear pressure quantitatively behind an intact drum for shorter or longer periods with an accuracy of ±0 6 cm H_2O Unfortunately some of the normal cases and most pathological ones cannot equilibrate their middle ear pressure completely because of reduced tubal function The neutral position of the drum cannot be recorded exactly and the volume of the middle ear cannot be calculated Middle ear pressure determination can therefore be made only after certain approximations in the calculation procedure If we analyse the accuracy at such an approximation we find that the pressure balance across the drum can be assessed with an accuracy of ±1 84 cm H_2O In cases with middle ear volumes less than 6 ml and with flaccid drums however the accuracy decreases

To be able to assess the indirectly determined normal middle ear pressure in normal persons, the tubal function must be investigated since we have found varying degree of normal function (Elner et al 1971) In all cases with perfect tubal function the middle ear pressure is kept close to the ambient pressure whereas in normal cases with a less good tubal function the range of the middle ear pressure is greater In many of these latter cases we know that for instance movement of the mandible blowing the nose or the Valsalva manoeuvre is used to ventilate the middle ear Determination of the middle ear pressure on normal cases is thus necessary for the determination of the normal pressure regulation in the middle ear a prerequisite for further analysis of pathological cases

ZUSAMMENFASSUNG

Beschreibung einer neuen Methode zur indirekten Bestimmung des Druckes im Mittelohr Im Prinzip werden die maximale Bewegungsgeschwindigkeit des Trommelfells und ihre Volumenveränderungen wahrend Schwankungen im Umgebungsdruck registriert Die Genauigkeit der Methode ist an Hand eines Kontrollmaterials analysiert worden Der Normaldruck im Mittelohr ist an 86 Personen mit unauffalligem Ohrenbefund studiert und mit der Tubenfunktion verglichen worden Bei guter Tubenfunktion halt sich der Druck im Mittelohr im Niveau mit dem Umgebungsdruck wahrend der Mittelohrdruck von Personen mit weniger guter Tubenfunktion grösseren Schwankungen unterworfen ist

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EFFICIENCY OF THE STENGER, DOERFLER STEWART AND LENGTHENED OFF-TIME BEKESY TESTS

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Abstract The fixed frequency Bekésy tracing was found to be an efficient indicator of nonorganic hearing loss when the off-time of the pulsed signal was lengthened from 200 to 800 msec The Lengthened Off Time (LOT Bekésy) Stenger and Doerfler Stewart (D-S) tests were administered when appropriate to 725 clinic patients The LOT Bekesy test correctly identified 96% of the 225 nonorganics vs 77% for the D-S test and only 48% for the Stenger test. Among 500 organically impaired patients the LOT Bekesy test, when applicable was 98% correct, the Stenger test was 86% correct and the D-S was 79% correct. In terms of applicability the LOT Bekesy test could be employed with 99% of the total 725 patients the Stenger test with 57% and the D-S test was appropriate with only 38% of the patients

Otologists and audiologists continue to report that a substantial number of their patients demonstrate some degree of nonorganic hearing loss Despite an awareness of the problem it is possible that some patients are misclassified because tests for nonorganicity are not routinely administered Ventry & Chaiklin (1965) in discussing tests of nonorganic hearing loss pointed to the need for more efficient and less time consuming procedures A rapid screening test was needed which would require minimal training for administration and interpretation of results It should have few limitations to its applicability while maintaining a high degree of accuracy

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The Lengthened Off Time (LOT Bekesy) test was recently developed in an attempt to meet the above criteria (Hattler 1970 Hattler & Schuchman 1970) The LOT Bekesy test employs a simple modification of the fixed frequency Bekesy tracing. Jerger & Herer (1961) first reported that nonorganic impairments result in the Type V Békesy pattern in which there appears to be a greater loss of hearing for pulsed than for continuous tones When the off time of the Bekesy s pulsed tones was lengthened from the conventional 200 msec to 800 msec while maintaining a 200 msec on time there was a remarkable exaggeration of the Type V separation for nonorganic patients Use of the lengthened off time pulse increased the Bekesy s efficiency for identification of nonorganicity to 95% from 40% with the conventional signals while there was no significant effect on the threshold tracings of organically impaired patients (Hattler 1970)

In this investigation the authors sought to compare the efficiency of the LOT Bekesy test with that of the two most commonly used screening tests for nonorganicity the Stenger & Doerfler Stewart (D-S) tests

Each of the three tests under investigation have limitations to their applicability The Stenger test requires a threshold asymmetry of approximately 15 dB or greater to insure reliable and valid results The D-S test presupposes a loss of hearing for spondee word

For the purposes of this investigation the D-S test was considered inappropriate unless the speech reception threshold (SRT) was greater than 30 dB (re 20-dB SPL) The LOT Bekesy test cannot be employed with patients who fail to respond to any pure tone at the maximum power output of the Bekesy audiometer (approximately 120-dB HL within the speech frequency range)

MATERIAL AND METHODS

The Stenger D-S and LOT Bekesy tests were administered when appropriate to 725 patients from a clinic population known to have a high incidence of nonorganicity The patients were classified as either organic or "nonorganic on the basis of results from standardized audiologic tests including pure tone and speech audiometry electrodermal skin response audiometry evoked cortical response and pure tone delayed auditory feedback tests The LOT Bekesy test for both ears at 1 000 Hz routinely preceded other tests The D S followed and the Stenger tests (speech and pure tone) were administered during the routine hearing evaluation The order of testing failed to have any noticeable influence on the efficiencies of the three procedures

Stenger test

The Stenger test was administered following the procedure recommended by Gaeth (unpublished paper) Each signal originated from a single source and one interrupter switch was employed The signal was split and applied inphase to the earphones at a sensation level of 5 dB to the better ear and 5 dB below volunteered threshold in the poorer ear If the patient failed to respond the Stenger test was considered positive for nonorganicity If the patient responded to the above condition the 5-dB SL signal was removed from the better ear If the patient continued to respond to the signal in the avowed poorer ear the test results were again considered indicative of nonorganic hearing loss

Doerfler Stewart test

The D S procedure was that of Doerfler & Epstein (Newby 1958) Spondaic words (PAL Auditory Test No 9) were presented live voice via a two-channel speech audiometer (Grason Stadler Model 162) The masker was a saw tooth noise with a base frequency of 128 Hz The D S results were considered positive if the patient exceeded the limits on any of three indicants (1) $SRT_1 - SRT$ -4 dB to 4 dB (2) SRT_{best} noise detection threshold (NDT) -4 dB to 16 dB (3) [$SRT_1 + 4$ dB] noise interference level (NIL) -10 dB to -40 dB These criteria were selected on the basis of unpublished data (Doerfler & Epstein 1956) and more than 20 years clinical experience at Walter Reed General Hospital

LOT Bekesy test

The instrumentation for the LOT-Bekesy test was modified during the course of the investigation. Initially the output of a conventional Bekesy audiometer (Grason Stadler Model 800-E) was routed in an electronic switch (Grason Stadler Model 829C) which was triggered by an interval timer (Grason Stadler Model 471 1) After leaving the output of the electronic switch the signal re-entered the Bekesy at the alternate in position The interval timer was set to deliver a pulsed tone of 200 msec on time and 800 msec off time The Bekesy was set to modulate the tone s intensity at a rate of 5 dB sec It was later found expedient to eliminate the need for external timing systems by recording the LOT pulsed and continuous signals on magnetic tape The signals were replayed via a tape recorder (Ampex Model 755) coupled to the alternate input of the Bekesy audiometer The results of these two approaches were indistinguishable and they were combined for this discussion Procedures for administration of the LOT Bekesy test are given below (1) The patient was instructed to press and hold the response switch as soon as he heard the sound and to release it when the sound was gone (2) The LOT pulsed tone was introduced to either ear at 0 dB and allowed to ascend

The patient was given approximately one minute for tracking (3) The signal was removed switched to the opposite earphone and allowed to ascend from 0 dB (4) The signal was re moved changed to the continuous mode and allowed to ascend from 0 dB (5) Finally the continuous tone was removed switched back to the original earphone and again allowed to ascend as in step 4

LOT tracings were considered positive for nonorganicity only if the continuous tracing suggested 5 5 dB or better hearing than pulsed tones Whenever there was a discrepancy between the initial and final proportions of the tracing the final portion was employed in the interpretation of results For purposes of classi fication the patient was considered to be non organic if either ear demonstrated LOT posi tive results

RESULTS

Stenger test

The Stenger test results shown in Table I, Box E indicate that 178 (approximately 35%) of the organic group could not take the test be cause of bilateral threshold symmetry Only 10 (2%) of the organic patients were mis classified by the Stenger test (Box B Table I) [illegible]ever the Stenger test had relatively poor [illegible]iciency among the nonorganic patients Nearly 60 Box F could not take the test and in Box D only 20% of the total non organic group were correctly identified by the Stenger test

Table I *Stenger test results among the total of 725 patients*

	[illegible] (A/C)	POSITIVE (B/D)	NOT APPLICABLE (E/F)	TOTAL
ORGANIC	A 312 (6[illegible] %)	0 (2 0%)	E 178 (35 6%)	500
NONORGANIC	C 48 ([illegible] 3%)	D 45 ([illegible]0 0%)	F 132 (58 7%)	225
TOTAL	360	[illegible]5	310	725

Table II *Stenger test results for only those pati ents who took the test*

	NEGATIVE	POSITIVE	TOTAL
ORGANIC	A 312 (96 9%)	B 10 (3 1%)	322
NONORGANIC	C 48 (51 7%)	D 45 (48 3%)	93
TOTAL	360	55	415

Table II represents only those patients for whom the Stenger test was applicable It can be seen that the test was remarkably efficient with the organic group Box A but it was less than 50% correct in identifying the nonorganic pa tients Box D Considering Boxes A and D 86% of the total patients who took the Stenger test were correctly classified

Doerfler Stewart test

The overall results of the D S test appear in Table III Among the 500 organic patients 301 (60% Box E) had SRT's better than 30 dB and the D-S test was not considered applicable Approximately 32% in Box A of the organic patients yielded D S negative results while 8% were misclassified by the test (Box B) A major limitation of the D S test was that nearly 65% of the nonorganic patients could not be tested

Table III *Doerfler Stewart test results among the total of 725 patients*

	NEGATIVE	POSITIVE	NOT APPLICABLE	TOTAL
ORGANIC	A 159 (3[illegible] 8%)	B 40 (8 0%)	E 301 (60 2%)	500
NONORGANIC	C 18 (8 0%)	D 61 (27 [illegible]%)	F 146 (64 9%)	225
TOTAL	177	101	447	725

Table IV *The Doerfler Stewart test results for only those patients who took the test*

	NEGATIVE	POSITIVE	TOTAL
ORGANIC	A 159 (79 9%)	B 40 (20 1%)	199
NONORGANIC	C 18 (22 9%)	D 61 (77 1%)	79
TOTAL	177	101	278

(Box F) Only 27 of the total nonorganic patients yielded positive D S results Box D

Table IV represents only those patients for whom the D-S test was appropriate While the D S test was 77% effective among the non organic patients Box D it yielded false positive findings in 20 of the organically impaired group in Box B Considering Boxes A and D Table IV the D S test had an overall efficiency of approximately 79 for both diagnostic groups

LOT Bekesy test

The LOT Bekesy test correctly classified 490 (98 Box A in Table V) of the organic group Only 3 patients (0 6%) failed to respond to pure tones at maximum intensity in both ears rendering the LOT test inapplicable Among the 225 nonorganic patients only one Box F

Table V *LOT Békesy test results among the total of 725 patients*

	NEGATIVE	POSITIVE	NOT APPLICABLE	TOTAL
ORGANIC	490 (98 0%)	B 7 (4%)	E 3 (0 6%)	500
NONORGANIC	8 (3 6%)	D 216 (96 0%)	F 1 (0 4%)	225
TOTAL	498	223	4	725

Table VI *LOT Bekesy test results for only those patients who took the test*

	NEGATIVE	POSITIVE	TOTAL
ORGANIC	A 490 (98 7%)	B 7 (1 3%)	497
NONORGANIC	C 8 (3 5%)	D 216 (96 5%)	224
TOTAL	498	223	721

could not be evaluated with the LOT test Positive LOT tracings were produced by 96 of the nonorganic group (Box D) while only 3 5 % (Box C) were missed by the test In Table VI Boxes A and D contain the patients who were correctly identified a total of 98 of all those tested

There were no consistent patterns in the audiologic profiles of those patients who were misclassified by the LOT test. Six of the 7 mis labeled organic patients had pathologies of the middle ear resulting in sizable air bone gaps The middle-ear muscle reflex was absent from all 7 patients The large majority of the pa tients with middle-ear pathology and absent muscle reflex produced negative LOT tracings Two of the 7 patients also yielded false posi tive D S test Only one patient yielded false positive LOT tracings in both ears

There was no characteristic profile for the 8 nonorganic patients who were missed by the LOT test The size of the nonorganic compo- nent at 1 000 Hz averaged 32 dB One patient yielded a false negative Stenger test.

The LOT Bekésy test, unlike the Stenger and D S tests allows the audiologist to evaluate each ear independently Eighteen of the patients with an average nonorganic component of 33 dB had a positive LOT test in only one ear despite the presence of bilateral nonorganic involvement Once again the audiologic profiles failed to provide clues as to the reasons for false negative

results The number of left and right ears missed by the LOT test were essentially equal The total of 26 nonorganic patients for whom the LOT test was erroneously negative in one or both ears comprised 11 6% of the entire nonorganic group

Administration time

The time required to administer these screening tests is an important clinical aspect The Stenger test was the quickest of the three, requiring an average of only 25 sec to administer The LOT test required an average time of 3 4 min whereas the D S test took more than 5 min to complete

DISCUSSION

The LOT Bekesy test takes advantage of a phenomenon in which a decrease of duty cycle (or percentage of time the sound is on) results in an SPL increase of loudness memory tracings (Hattler 1968) The conventional Bekesy test compares the tracings of a continuous tone 100% duty cycle with that of a pulsed tone of 50% duty cycle In the LOT Békésy test the tracing of a pulsed tone with on times of 200 msec and off times of 800 msec (20% duty cycle) is compared to the continuous tracing The difference in duty cycle is greater in the LOT Bekesy test than in conventional fixed [illegible]y Békésy tests Consequently the LOT test yields greater Type V separation and improves the efficiency of the test in the identification of nonorganic patients

When the three screening tests were ranked as to overall efficiency the LOT Bekesy test had the highest accuracy at 98% followed by the Stenger test at 86% and the D S test at 79% In terms of applicability the LOT test could be used in 99 5% of the total patients whereas the Stenger test could be used in only 57% of the patients and the D S in only 38% When applicable the Stenger test yielded the poorest efficiency among nonorganic patients labeling only one half the patients correctly These findings are in excellent agreement with the results of Ventry & Chaiklin (1965) who reported that the Stenger test was appropriate in 55% of their patients with an efficiency of only 43% when applicable A typical patient with a false negative Stenger test had a unilateral organic loss of hearing with nonorganic overlay in both the impaired ear and the organically normal ear

Several false D S positive results were observed on organic patients with conductive lesions In this situation the most common positive finding was a noise interference level greater than 40 dB above SRT_I Another common observation was the noise detection threshold more than 16 dB below the SRT resulting in a false positive D S test in patients who had a sharply sloping pure tone audiometric configuration It is difficult to compare the present results on the D S test with those of Ventry and Chaiklin primarily because of the large difference in patient sample size and because somewhat different test criteria were employed Use of the Walter Reed criteria for D-S positive findings resulted in the reduction of test efficiency among organic patients

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Josephine Walker Charlene Scherr and Earl Brown provided assistance in data collection Portions of this manuscript were presented at the 46th Annual Convention of the American Speech and Hearing Association New York November 1970

ZUSAMMENFASSUNG

Der Békésy Test mit konstanter Frequenz stellte sich als ein wirkungsvoller Indikator des nichtorganischen Gehorsverlustes dar wenn man die Unterbrechungsperiode des pulsierenden Signals von 200 auf 800 m sec verlangerte Diese Modifikation bezeichnen wir als den LOT Békésy Test Dieser Test sowie die Stenger und Doerfler Stewart Teste wurden an 7?5 geeigneten klinischen Patienten durchgefuhrt Der LOT Békésy Test identifizierte 96% der Doerfler Stewart Test 77% und der Stenger Test lediglich 48% der Fälle von nichtorganischem Gehorsverlust Unter den organisch beeintrachtigten Patienten ergab der LOT Békésy Test in anwendbaren Fallen 98 korrekte Ergebnisse der Stenger Test 86 und der Doerfler Stewart Test 79% Der LOT Békésy Test konnte an 99% der Patienten vorgenommen werden der Stenger Test an 57% und der Doerfler Stewart Test war nur bei 38% der Patienten anwendbar

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results The number of left and right ears missed by the LOT test were essentially equal The total of 26 nonorganic patients for whom the LOT test was erroneously negative in one or both ears comprised 11 6% of the entire non organic group

Administration time

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and brain the enzyme has been shown to be controlled by adenosine triphosphate (ATP) and citrate (Lowry & Passonneau 1966) The role of PFK becomes even more important in bone since the substrate fructose-6-phosphate for this enzyme also serves as a precursor in the synthesis of the ground substance in bone

The central role of PFK in glycolysis and in ground substance synthesis has led to the investigation described in this report The activities of PFK have been determined in otosclerotic and non otosclerotic bone The control of PFK by ATP and citrate has been compared in muscle and bone

MATERIAL AND METHODS

Stapedes were obtained from patients with clinically diagnosed otosclerosis confirmed at surgery Stapedes incudes and mallei removed at autopsy from non-otosclerotic individuals were used as controls Surgical samples were quickly rinsed in Ringers lactate solution to remove any adhering blood The specimens were then blotted well to prevent premature extraction wrapped in foil quick frozen and stored

The stapedes and other ossicles were ground in the frozen state with a mortar and pestle on dry ice The fine homogeneous powder was extracted with a 0 3 M potassium phosphate buffer pH 8 0 which contained 0 003 M ethylenediaminetetraacetic acid (EDTA) and 0 003 M potassium fluoride The EDTA and fluoride were included to provide maximum stability of phosphofructokinase (Ling et al 1965)

Extraction was accomplished in the cold for 15 hours by suspending the bone powder in the extracting medium at a concentration of 100 mg/ml for stapedes and 200 mg/ml for other ossicles The suspension was stirred occasionally and then allowed to stand overnight The following morning the suspension was again mixed and then centrifuged at 2 000 g for 5 min The resulting supernatant fluid was carefully removed with a pasteur pipette to exclude bone particles and placed in another tube The bone residue was resuspended in extracting buffer equal to the amount used in the first extraction and allowed to stand in the cold for 30 min with occasional stirring After centrifugation this supernatant was combined with the initial supernatant resulting in extracts with concentrations of 50 milligrams of bone per milliliter of extract for stapedes and 100 milligrams of bone per milliliter of extract for other ossicles The extracts were assayed for protein and phosphofructokinase activity

Protein concentrations were determined colorimetrically (Lowry et al 1951) Crystalline bovine albumin was used as the protein standard

Phosphofructokinase activity was measured by coupling the reaction (F-6 P + ATP $\xrightarrow{PFK}$ F 1 6 diP + ADP) with the auxiliary enzymes[1] aldolase triosephosphate isomerase and alpha glycerophosphate dehydrogenase The rate of oxidation of dihydronicotinamide adenine dinucleotide (NADH) in the alpha glycerophosphate dehydrogenase reaction was determined spectrophotometrically in a recording spectrophotometer

The standard assay for phosphofructokinase contained the following in a total volume of 3 ml 33 mM Tris (hydroxymethylaminomethane hydrochloride (Tris HCl) buffer pH 8 0 containing 2 mM $MgSO_4$ and 6 6 mM cysteine HCl 0 1 mM NADH 0 33 mM F-6 P 0 33 mM ATP 0 1 ml auxiliary enzyme solution and bone extract (0 2–0 3 ml) or solution of crystalline PFK (0 01 ml) The auxiliary en

[1] The following enzyme preparations were obtained from Sigma Aldolase 1 mg converts 8 7 micromoles F 1 6 diP per min at pH 7 4 at 25 C triosephosphate isomerase 1 mg yields 40–80 micromoles glyceraldehyde 3 phosphate per min at pH 7 6 at 25 C alpha glycerophosphate dehydrogenase 1 mg reduces 60–120 micromoles dihydroxyacetone phosphate per min at pH 7 4 at 25 C phosphofructokinase (ATP D fructose-6 phosphate 1 phosphotransferase EC 2.7 1 11) 1 mg converts 60 micromoles of F 6-P per min at pH 8 0 and 37 C This crystalline preparation was diluted 1 1 000 with extraction buffer containing 2 mg per ml of crystalline bovine serum albumin. F 1 6-diP fructose 1 6 -diphosphate ADP adenosine diphosphate F 6-P fructose 6-phosphate

zyme solution contained 1 25 mg crystalline aldolase 1 mg alpha glycerophosphate dehydrogenase and 0 5 mg triosephosphate isomerase in 5 ml of 33 mM Tris HCl buffer pH 8 0 containing 2 mM $MgSO_4$ 6 6 mM cysteine hydrochloride and 2 mg per ml of crystalline bovine serum albumin All reactants except the enzyme solutions were maintained at 38°C during the preparation of the reaction The reaction was initiated by the addition of fructose 6 phosphate The reference cell contained all constituents except fructose 6-phosphate The change in absorbance was measured at 340 nm at 37 C for 20 min

RESULTS

The validity of the coupled PFK reaction was determined using crystalline PFK and extracts of incudes and mallei The auxiliary enzymes were determined to be non rate limiting by establishing that no increase in velocity resulted when greater amounts of auxiliary enzymes were added The efficacy of the auxiliary enzymes were established further by demonstrating that the velocity of the overall reactions remained proportional to added PFK up to changes of absorbance of 0 1 per minute ll subsequent reactions were run at velocities esulting in absorbance changes below this rate

PFK in ossicles

A comparison of the PFK activity of otosclerotic stapedes and non otosclerotic stapedes mallei and incudes is shown in Table I Protein content of each of the ossicles is also included It was observed that PFK activity and protein concentration of otosclerotic and non-otosclerotic stapedes are the same The overall enzyme rates also remain the same when glucose-6-phosphate is substituted for fructose 6 phosphate as the substrate in the reaction The PFK activity and protein concentration is higher however in both the disease and non disease stapedes than in the outer two types of ossicles This observation is consistent with a previous report which noted a higher metabolic activity in stapedes than in mallei and incudes (Soifer et al 1967 Soifer et al 1969)

Table I *Phosphofructokinase activity in ossicles*
M = mean S E = Standard error of the mean
n = Number of pools

Tissue	Protein[a]	PFK[b]	
Mallei pool[c] ($M \pm$ S E)	9 9 ± 0 33	0 71 ± 0 066	$n=9$
Incudes pool ($M \pm$ S E)	7 0 ± 0 17	0 97 ± 0 072	$n=6$
Non-otosclerotic stapedes pool			
1	15 2	1 58	
2	11 7	2 64	
3	21 6	1 32	
Otosclerotic stapedes pool			
1	21 4	1 22	
2	23 4	1 26	
3	20 8	1 44	
4	17 8	1 52	

[a] Expressed as micrograms of protein per milligram wet weight of bone
[b] Expressed as $\times 10^{-4}$ micromoles of substrate converted per min per microgram of protein
[c] Contains bone tissue from 15–20 patients

Control of PFK by ATP

Phosphofructokinase activity in muscle liver and brain is controlled by ATP The effect of varying concentrations of ATP on bone and muscle phosphofructokinase is compared in Fig 1 Regardless of the ossicle used no inhibition of phosphofructokinase was observed at concentrations of ATP which were demonstrated to effectively inhibit crystalline PFK from muscle In fact at concentrations of ATP which nearly inhibit the muscle enzyme completely the PFK from bone has yet to reach maximum velocity

It was observed however that when ATP was omitted the rate of the PFK reaction was lower in the otosclerotic stapedes (4×10^{-6} micromoles of substrate converted min^{-1} microgram protein^{-1}) than in the control stapedes (8×10^{-6} micromoles of substrate converted min^{-1} microgram protein^{-1})

This difference in PFK activity was not observed however under the standard condi

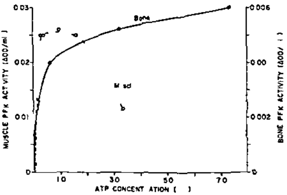


Fig 1 The effect of increasing concentration of ATP on phosphofructokinase activity from muscle and bone The standard PFK assay was used except that ATP concentrations were changed to those indicated Extracts of stapedes were used as a source of bone PFK Similar curves were obtained using extracts of mallei

tions for the assay of the PFK or under conditions in which the ATP concentrations were increased and were sufficient to inhibit the muscle enzyme

Control of PFK by citrate

Citrate an intermediate of the tricarboxylic acid cycle also exerts a control on the rate of glycolysis since it has been shown to be an inhibitor of phosphofructokinase This has been demonstrated in skeletal muscle heart brain and liver The importance of this control function of citrate on PFK was evaluated in osseous tissue with particular interest and emphasis on a comparison between otosclerotic and normal bone tissue

A comparison was first made of the effect of citrate on bone (mallei) and muscle PFK at two concentrations of ATP The results are shown in Fig 2 The two concentrations of ATP used were 0 33 mM which was optimum for muscle PFK and 3 33 mM which caused a 50 inhibition (Fig 1) All PFK rates are compared to the rate in the absence of added citrate At 0 33 mM ATP and 0 1 mM citrate the bone PFK was increased whereas muscle PFK was inhibited to 80% of the activity noted in the absence of citrate No further changes in the activity of the bone enzyme were observed at higher concentrations of citrate but the muscle enzyme was inhibited to 60 of the rate observed in the absence of citrate

At the higher ATP concentration (3 33 mM) the muscle PFK was inhibited to 60° at 0 1 mM citrate and to 12 at 1 0 mM citrate The bone PFK also becomes susceptible to inhibition by citrate at the higher ATP concentration The degree of inhibition (to 80) was considerably less than the inhibition of muscle PFK at 0 1 mM citrate however the inhibition was not appreciably greater at 1 0 mM citrate as was observed with muscle PFK

The effect of citrate on PFK in extracts of otosclerotic and non otosclerotic stapedes at three concentrations of ATP is shown in Fig 3 At endogenous concentrations of ATP (no ATP added to reaction) an increase in PFK activity is observed with increasing citrate concentrations in both otosclerotic and control tissues The increase is not as great in the otosclerotic however At 0 67 mM ATP no effect of citrate on PFK activity was observed in otosclerotic bone while the PFK in the control was inhibited with increasing citrate A similar effect of citrate on PFK activity is

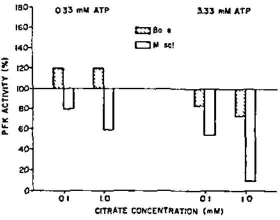


Fig 2 The effect of citrate on phosphofructokinase activity from muscle and bone The standard assay was used for PFK except that ATP concentration was *increased to 3 33 mM as indicated Extracts of mallei* were used as source of bone PFK The PFK activity obtained with no citrate was set at 100% all PFK activities in the presence of added citrate were compared to the activity in the absence of citrate

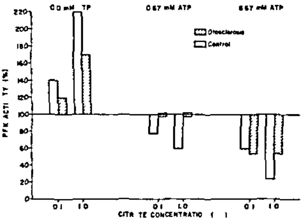


Fig 3 The effect of citrate on phosphofructokinase activity from otosclerotic and non otosclerotic stapedes The standard assay was used for PFK except that *ATP concentrations were changed to those indicated* The activity obtained with no citrate was set at 100 per cent all PFK activities in the presence of added citrate were compared to the activity in the absence of citrate

observed at 6 67 mM *ATP* but with an overall increased inhibition in both otosclerotic and controls The response of the PFK from bone at this ATP concentration was quite similar *to that noted with muscle PFK at 3 33 mM* ATP (Fig 2)

Using G-6 P did not alter PFK activities observed in either otosclerotic or non-otosclerotic bone at 0 67 mM ATP Citrate had the same inhibitory effects on these rates

DISCUSSION

Phosphofructokinase activity determined under optimum conditions was found to be the same in otosclerotic and non otosclerotic bone (Table I) Glucose 6 phosphate was substituted for fructose-6-phosphate as substrate in the reaction in an attempt to ascertain if the rate would decrease because of (1) a diversion of the greater amounts of fructose 6-phosphate to other pathways (2) the increased alkaline phosphatase found in otosclerotic bone was of sufficient concentration to hydrolyze a sufficient amount of fructose-6 phosphate so the PFK rate would be decreased

Under these altered conditions no decrease in PFK activity was noted (Table I) First in the system used this indicated that hexose isomerase was not limiting Secondly it suggested that sufficient amounts of F 6 P were not being hydrolyzed or channeled into other pathways to effect the PFK rate in either of the bone tissues The possibility also exists, however that if the substrates remained bound to the enzymes during the course of reaction that fructose 6-phosphate may not have been available for other reactions

When phosphofructokinase was determined in the stapedes in the absence of added ATP it was noted that rates were decreased in both tissues but that the rate was lower with otosclerotic bone than with normal bone The available ATP may be less in otosclerotic bone but actual concentrations have not been determined

The higher rates of PFK activity observed in the stapedes compared to mallei and incudes is consistent with higher metabolic activity of stapedes which has been noted previously with other enzymes (Soifer et al 1967 1969)

This may be as suggested an inherent property of the tissue without further significance On the other hand it may be one of the predisposing factors for the development of otosclerotic foci in the stapedes as opposed *to other ossicles*

The observation that PFK from osseous tissue responds to control by ATP differently than muscle PFK suggests that a different *form of this enzyme may exist in bone A difference in the degree of purification of* the PFK from the two sources may account for the observation but such a possibility is difficult to resolve because of the limited availability of ossicles as a source of the enzyme Another possibility for the observed effect of ATP on bone could be due to some artifactitious constituent(s) obtained as a result of the extraction process In an attempt to resolve these possibilities muscle PFK was added to a reaction containing bone extract The resulting activity was equal to the sum of the separate activities Thus crystalline enzyme was unaffected by possible constituents inherent to the bone extract and the difference in the

effect of ATP on PFK from muscle and bone appears valid

While it appears that the control of PFK by ATP in bone is different from that observed in muscle the control (inhibition) by citrate is observed in bone (Fig 2) as well as muscle The effect varies with concentrations of ATP present in the reaction and at 3 33 mM ATP the enzyme in both bone and muscle is inhibited to different degrees This difference in degree of sensitivity to citrate was observed between otosclerotic and non-otosclerotic stapedes as well In the absence of added ATP no inhibition is noted by citrate in fact there is an increase in activity with increasing amounts of citrate added At higher concentrations of ATP the normal bone tissue is more sensitive to citrate inhibition and control

A citrate site and a synergism between citrate and ATP has been reported for skeletal muscle PFK (Lowry & Passonneau 1966) We observe a similar synergism between citrate and ATP from PFK in bone (ossicles)

The results indicate that PFK from otosclerotic stapedes is more insensitive to control by citrate than PFK from non-otosclerotic stapedes This suggests that in otosclerosis glycolysis is not as well regulated by ATP and citrate

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ZUSAMMENFASSUNG

Die Aktivitat der Phosphofruktokinase wurde in Gehorknochen Extrakten von Patienten mit und ohne Otosklerose bestimmt. In anderen Versuchen wurde die Wirkung des Adenosintriphosphates und des Zitrates auf die Aktivitat der Phosphofruktokinasen der Gehorknochelchen und besonders des Steigbugels bei Patienten mit Otosklerose untersucht Adenosintriphosphat Konzentrationen, die gewohnlich die Phosphofruktokinasen von Muskel Herz Leber und Gehirn Geweben hemmen hatten keine Wirkung auf das Enzym von Gehorknochelchen. Die Aktivitat der Phosphofruktokinase ist normalerweise grösser in den Steigbugeln als in den anderen Gehorknochelchen die Aktivitat otosklerotischer Steigbugel ist jedoch in etwa gleich der bei normalen Gehorknochelchen Die Phosphofruktokinase otosklerotischer Steigbugel reagiert anders das ist in geringerem Masse als bei normalen Kontrollen auf die steigende Konzentrationen von Zirat zuruckzufuhren Glukose 6-Phosphat an Stelle des Fruktose 6-Phosphates im Phosphofruktokinasen Reaktionsgemisch ist der Aktivitat die man mit Fruktose 6-Phosphat erzielt ahnlich Man kann daher annehmen dass die Hexoseisomerasen im Gewebe von Patienten mit oder ohne Otosklerose etwa die gleichen Aktivitaten haben Weiterhin wurde in Experimenten mit Glukose 6-Phosphat als Substrat kein Unterschied in den Reaktionsgeschwindigkeiten gefunden das deutet an dass genügend Substrat für eine optimale Phosphofruktokinase Aktivitat bei Patienten mit Otosklerose vorhanden ist. Die oben beschriebenen Abnormalitaten werden zu fruher aufgezeigten Aktivitats-Veranderungen der Milchsaure Dehydrogenase und der alkalischen Phosphatase in Beziehung gesetzt.

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STUDY OF THE ACTION POTENTIAL IN THE ISOLATED POSTERIOR AMPULLAR RECEPTOR

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Abstract The posterior semicircular canal of frog was isolated to lead potential from the ampullar nerve The ampullar receptor was stimulated with a device which could induce both ampullofugal and ampullopetal flows By using an a c amplifier it was discovered that the posterior ampullar receptor was effectively stimulated by ampullofugal flow resulting in an increase in nerve impulse frequency while an ampullopetal flow caused no response When a d c amplifier was used to record responses on similar stimulation an ampullofugal stimulation caused a positive slow potential whereupon an increase in nerve impulse frequency was observed Whether or not this potential was in truth a biologic one was investigated utilizing Ba ion Pronounced influence from the Ba^{++} ion showed that it was evidently a biologic one

In the field of fundamental investigation of the vestibulum most problems concerning the nerve periphery appear to have been solved and interest is now focused on the centrum However the mechanism of the peripheral vestibular receptor cannot be regarded as completely resolved

From this standpoint the author for the purpose of clarifying the function of a single semicircular canal has isolated the posterior semicircular canal of frog stimulated it by an experimental deviation of the cupula and obtained potentials from the ampullar nerve

In this paper it is reported that positive and negative d c potentials have been obtained by ampullofugal and ampullopetal deviation of the cupula The characteristics of these potentials are pursued

METHOD

Under microscopic control the posterior semicircular canal of frog (*Rana esculenta*) is isolated in Tyrode solution with great care not to injure the ampullar nerve A fine glass pipette is introduced into the posterior end of the isolated canal The pipette is connected to a micrometer syringe filled with Tyrode solution The device is arranged so that a rotation of a few degrees of this micrometer syringe facilitates aspiration or pressure of the fluid in the ampulla (Fig 1) By manual rotation to the right 0 15 μl of fluid is aspirated ampullofugally whereas by a rotation to the left the same amount of fluid is compressed ampullopetally Both are utilized as stimuli

In the meantime potentials are picked up by a suction electrode (Rapuzzi & Casella 1965) from the ampullar nerve and observed with a Braun tube oscilloscope To represent the number of spikes appearing in response to an ampullofugal stimulation spikes over 50 μV are calculated for a 7 sec period with an electronic counter A d c amplifier is used in order to observe the leading of d c potentials

For about 20 min after the isolation of the semicircular canal the number of spikes as well as the amplitude of d c potentials was variable Therefore each isolated semicircular canal was used for experiment after a delay of at least 20 min

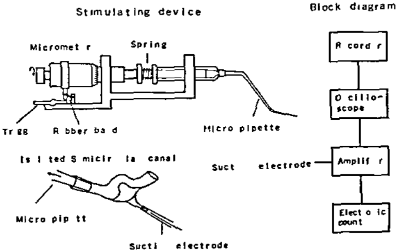


Fig. 1.

To test whether or not the positive and negative d.c. potentials appearing in response to an ampullofugal or an ampullopetal stimulation were an artefact, 0.25 ml of 1, 12, 18 and 25 mM/l $BaCl_2$ solution respectively was injected into the ampulla of the isolated semicircular canal preparate, and a stimulus was given one min after injection, the response being observed with the d.c. amplifier. This observation of the response to the stimulus was repeated six times with a 10-min interval.

RESULTS

After resting the isolated posterior semicircular canal in Tyrode solution for 20 min, the micropipette of the stimulating device was connected to the severed posterior end of the canal. When the micrometer was rotated 5 degrees to the right the fluid in the ampulla was aspirated ampullofugally, producing a stimulus. On recording with an a.c. amplifier approximately 1,500 spikes over 50 μV appeared within a 7 sec period (Fig. 2). In the meantime, under d.c. amplifier observation, a positive d.c. potential measuring 400–600 μV appeared. Furthermore, an increase in the nerve impulse frequency over this potential was noticed (Fig. 3).

On the other hand, on rotation to the left the fluid in the ampulla was compressed, stimulating the cupula ampullopetally. This time a negative potential was observed with a d.c.

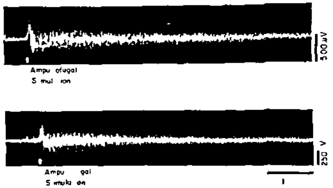


Fig. 2. The response of the posterior ampullar receptor.

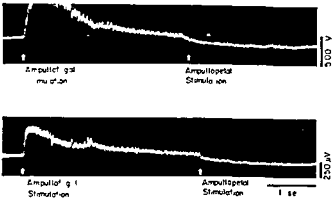


Fig 3 Slow potential from the posterior ampullar receptor

amplifier and the nerve impulse frequency was decreasing (Fig 3)

For the purpose of testing whether these d c positive and negative slow potentials are in fact a biologic potential the Ba $^{+}$ ion which is said to inhibit receptors was applied directly to the posterior semicircular canal receptor

0 25 ml of 25 18 12 and 1 mM/l BaCl solution respectively was injected into the ampulla within 30 sec and the variation of the d c slow potentials in response to the stimulation was observed

Injection of 25 mM/l BaCl was immediately followed by a complete loss of the slow poten observed with the d c amplifier as well as an increase in the nerve impulse obtained through the a.c amplifier as a response to the stimulation Sixty minutes of observation gave no sign of recovery of potential (Table I *a*)

Following injection of 18 mM/l BaCl positive and negative slow potentials appearing in response to stimulation showed a marked lowering and in the case of a c leading a decrease in the number of the spikes appearing in response to the ampullofugal stimulation was observed (Fig 4)

Fig 5 shows the influence of 18 mM/l BaCl on the amplitude of positive slow potential which appears when an ampullofugal stimulation is given Table I *b* shows the variation of the amplitude of the positive slow potential due to 18 mM/l in five cases Initial activity was 300–500 μV in amplitude and after BaCl solution injection a complete block of response

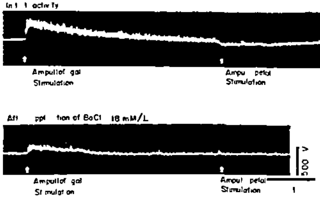


Fig 4 Slow potential from the posterior ampullar receptor

Table I *The influence of $BaCl_2$ on slow potential (SP) from the isolated posterior ampullar receptors of the frog*

SV Isolated semicircular canal
SP Amplitude of slow potential

SC	SP (μV)	Change in SP (μV) following application of BaCl after						
		Minutes						
		1	10	20	30	40	50	60
(a) BaCl concentration 25 mM/l								
1	500	0	0	0	0	0	0	0
2	600	0	0	0	0	0	0	0
3	400	0	0	0	0	0	0	0
4	400	0	0	0	50	50	0	0
5	300	0	0	0	0	0	0	0
(b) BaCl concentration 18 mM/l								
1	400	0	0	0	100	100	100	0
2	600	0	0	0	0	0	0	0
3	500	0	100	100	100	100	100	100
4	300	0	0	0	100	0	0	0
5	400	100	100	100	100	100	100	100
(c) $BaCl_2$ concentration 12 mM/l								
1	600	100	100	200	200	200	200	200
2	400	200	200	200	200	200	200	200
3	600	0	0	100	100	100	100	100
4	400	0	100	100	100	100	100	100
5	500	100	200	200	200	200	200	200
(b) $BaCl_2$ concentration 1 mM/l								
1	400	400	400	400	400	400	300	300
2	300	300	300	400	300	300	300	300
3	600	00	500	500	500	500	400	400
4	600	600	600	500	500	500	400	500
5	500	500	500	500	500	400	500	400

to an ampullofugal stimulation was observed in some cases but in others potentials as high as 100 μV could be still observed (Table I *b*)

Following injection of 12 mM/l BaCl solution preparates which had shown 400–600 μV initial activity of d c potential in response to the ampullofugal stimulation marked suppression of potential was exhibited ranging from a complete disappearance of the response to a lowering of the potential to 100–200 μV as shown in Table I *c*

Fig 6 shows variation of the slow potentials before and after 12 mM/l BaCl solution injection It is clearly seen that both positive and negative potentials were influenced It has been surmised further that the negative potential is more sensitive to Ba ion than the positive one

In a c amplifier observation giving ampullofugal stimuli a decrease in nerve impulse frequency was recognized (Fig 7)

When 1 mM/l BaCl solution was injected the influence on the positive and negative d c potentials was scarcely noticed indicating that these potentials could be influenced only by higher concentrations of BaCl solution (Table I *d*)

Solutions of Streptomycin and Kanamycin also weakened these potentials These facts might be regarded as evidence that they are not an artefact but a biologic potential which is induced by ampullofugal or ampullopetal deviation of the cupula

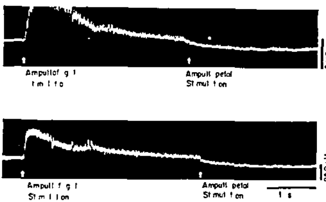


Fig 3 Slow potential from the posterior ampullar receptor

amplifier and the nerve impulse frequency was decreasing (Fig 3)

For the purpose of testing whether these d c positive and negative slow potentials are in fact a biologic potential the Ba⁺⁺ ion which is said to inhibit receptors was applied directly to the posterior semicircular canal receptor

0 25 ml of 25 18 12 and 1 mM/l BaCl solution respectively was injected into the am pulla within 30 sec and the variation of the d c slow potentials in response to the stimula tion was observed

Injection of 25 mM/l BaCl was immediately followed by a complete loss of the slow poten observed with the d c amplifier as well as an increase in the nerve impulse obtained through the a c amplifier as a response to the stimulation Sixty minutes of observation gave no sign of recovery of potential (Table I *a*)

Following injection of 18 mM/l BaCl posi tive and negative slow potentials appearing in response to stimulation showed a marked lower ing and in the case of a c leading a decrease in the number of the spikes appearing in re sponse to the ampullofugal stimulation was ob-served (Fig 4)

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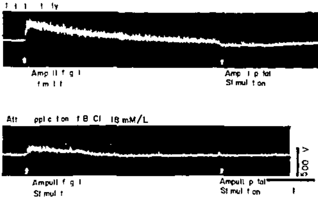


Fig 4 Slow potential from posterior ampullar receptor

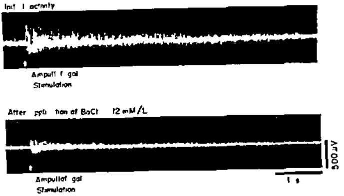


Fig 7 The response of the posterior ampullar receptor

circular canal a stimulation is carried by an ampullofugal current of endolymphatic fluid when an ampullofugal stimulation through aspiration is given to an isolated posterior semicircular canal in Tyrode solution the frequency of the nerve impulse is increased but is not stable immediately after the excision (Harada et al 1967 Harada 1968) The number of spikes counted within a 7 sec period following a single stimulation becomes stable approximately 20 min after the isolation This fact might possibly be interpreted as follows a sudden replacement of the endolymphatic fluid in the isolated semicircular canal by Tyrode solution i e an abrupt change in the composition of the endolymphatic fluid makes a stable depolarization impossible In the acoustico-lateralis system a unique situation exists viz. the endolymph facing the hair bearing end of the sensory cells has a high concentration of K ions which approaches that of the interior of the cells (Smith et al 1954) This is true also for the lateral line canal organ (Flock & Wersall 1964) Moreover it is widely recognized that K ions are closely related to the transducing mechanism of the inner ear However some authors have reported that in amphibia and birds the K ion concentration in the endolymphatic fluid is not so high

In this series of experiments utilizing isolated preparates where both the endolymph and the perilymph consist of Tyrode solution the author found an elevation of the discharge rate following ampullofugal stimulation It is therefore surmised that the concentration of K^+ ions in the endolymph of the frog semicircular canal is not high A further study is required on this point

As stated above the semicircular canal receptor is a mechanoreceptor From this fact many authors have predicted that mechanical stimulation ampullofugal and ampullopetal would cause an alteration in the d c potential

Flock (1965) in his investigations on the lateral organ of fish has reported that a depolarization i e excitation occurs when the stereocilia undergoes a displacement towards the kinocilium whereas a hyperpolarization i e inhibition occurs when the condition is opposite

The author has produced experimentally a cupular displacement by ampullofugal aspiration and ampullopetal compression of the content in the isolated posterior semicircular canal of frog A d c slow potential as shown in Fig 3 has constantly been obtained from each preparate through a d c amplifier

Following an ampullofugal stimulation a positive large potential of the rate of rise as large as 500 μV was obtained On this potential an increase in the nerve impulse frequency was also marked. When an ampullopetal stimulation was given as the positive potential approached the baseline a negative potential was obtained This potential resembles the generator potential which Gray & Sato (1955)

obtained in their experiment on the Pacinian corpuscles The increase in the nerve impulse frequency on an ampullofugal stimulation is suggestive of a depolarization whereas the de crease in the discharge rate on an ampullopetal stimulation suggests a hyperpolarization

To test whether these potentials were in truth an artefact or not Ba $^{+}$ ion which is considered to inhibit receptors directly (Ra puzzi et al 1961) was injected into the am pulla at 25 18, 12 and 1 mM/l concentration The d c potential as well as the discharge rate of the nerve impulse was lowered in proportion to the Ba^{++}-ion concentration Therefore these *potentials are not artefact but are doubtless bio* logic potentials which can be interpreted as being induced by mechanical displacements of the cupula

However this d c slow potential could not at once be considered to be identical with the generator potential which according to Gray & Sato (1955) originates in dynamic distortion of the unmyelinated part of the sensory nerve ending It is because of the leading method employed by the author not directly from the sensory cells but from the nerve ending of the ampulla However as the potential was ob tained through the ampullar nerve and aspi rated into the suction electrode from a posi tion very close to the sensory cells of the ampulla it is supposed possible that the gen erator potential originating in a mechanical displacement of the cupula was recorded with a d c amplifier

Concerning this potential nothing other than the above mentioned supposition can be said The author is now making an attempt to obtain directly the d c resting potential by introducing a microelectrode into the surface of the cupula together with further investigations on the trans ducing mechanism and the characteristics of the potential

ZUSAMMENFASSUNG

D r hintere Bogengang des Frosches wurde isoliert um Potentiale vom Ampullarnerv zu leiten Der am pullare Rezeptor wurde mittels eines Apparates ge reizt der beide ampullofugalen und ampullopetalen Strom ausmachen kann Mit Hilfe eines AC Ampli fikators wurde bemerkt dass der hintere ampullare Rezeptor mit einem ampullofugalen Strom effektiv gereizt wurde und es zu einer Vermehrung der Nervenimpulshaufigheit kam Auf einen ampullope talen Strom wurde keine Reaktion nachgewiesen Wenn ein DC Amplifikator gebraucht wurde erz ugte eine ampullofugale Reizung ein positives Potential auf welchem eine Vermehrung der Nervenimpulsfrequenz bemerkt wurde Demgegenuber wurde ein negatives Potential auf eine ampullopetale Reizung gefunden auf welchem eine Verminderung der Nervenimpuls frequenz bemerkt wurde Dass dieses Potential wirk lich ein biologisches war wurde mittels Ba^{++} ion gepruft Das wurde von Ba^{++} ion deutlich beeintrach tigt also kann es fur ein biologisches Potential ange sehen werden

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ACETYLCHOLINESTERASE ACTIVITY IN THE PERIVASCULAR NERVE PLEXUS OF THE BASILAR AND LABYRINTHINE ARTERIES

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(Received July 9 1970)

Abstract Acetylcholinesterase (AChE) activity was demonstrated in the perivascular nerve plexus of the cat basilar and labyrinthine arteries using a histochemical technique The basilar artery was found to be surrounded by dense AChE active nerve bundles and fibers The periarterial nerve networks were observed to continue on the anterior inferior cerebellar and labyrinthine arteries but under the light microscope none of the AChE positive nerve fibers were seen on the peripheral cochlear and vestibular arteries Though the vascular wall of the vessels in the stria vascularis and spiral ligament showed AChE activity neural structures were not discerned Since the perivascular innervation with AChE positive nerve fibers was detected only up to the labyrinthine and common cochlear arteries the neurogenic control of the blood vessels in the inner ear is possibly not directly exerted in arteries of the labyrinth

There are many clinical syndromes whose etiology is frequently blamed on angiospasm angioneurosis or vascular occlusion of the arteries in the inner ear Adrenergic innervation of these vessels has been well studied while little attention has been paid to the cholinergic system In the present paper the acetylcholinesterase (AChE) activity in the periarterial nerve plexus of the basilar and labyrinthine arteries was studied in order to elucidate the cholinergic control of the inner ear blood circulation

The author believes that the present study is the first to demonstrate convincing morphological evidence of cholinergic innervation in the basilar and labyrinthine arteries

MATERIAL AND METHODS

Five cats were used for this experiment After intraperitoneal anesthesia with sodium pentobarbital 30 mg/kg body weight thoracotomies were performed and the animals were perfused through the aorta with ice cold physiological saline solution for 10 min Animals were then perfused with 10 formol calcium solution of pH 7 1 at 4°C for 10 min and washed with cold physiological saline solution The prefixed animals were decapitated and the brains and temporal bones were removed

The preparation of the basilar and cerebellar arteries in toto

The basilar and cerebellar arteries were carefully removed *in toto* from the brain under the dissecting microscope and then transferred to the substrate solution for the demonstration of AChE activity The substrate solution was prepared after Karnovsky & Roots (1964) After being incubated *in toto* at 37 C for 90 min the tissues were briefly washed with distilled water and mounted on microscopic slides with PVP (Polivinyl Pyrrolidone) solution

AChE activity in the frozen section

In one cat the brain was submitted to frozen section with the basilar artery attached

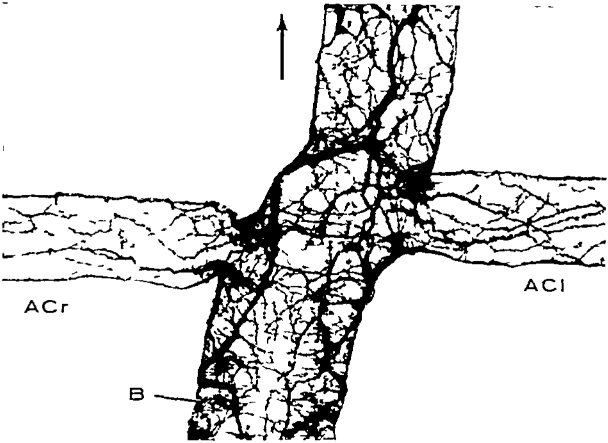


Fig 1 The basilar artery AChE activity in the nerve plexus around the basilar artery (*B*) The nerve net work is also seen in the right and left anterior inferior cerebellar arteries (*ACr ACl*) (arrow points to the anterior direction) × 43

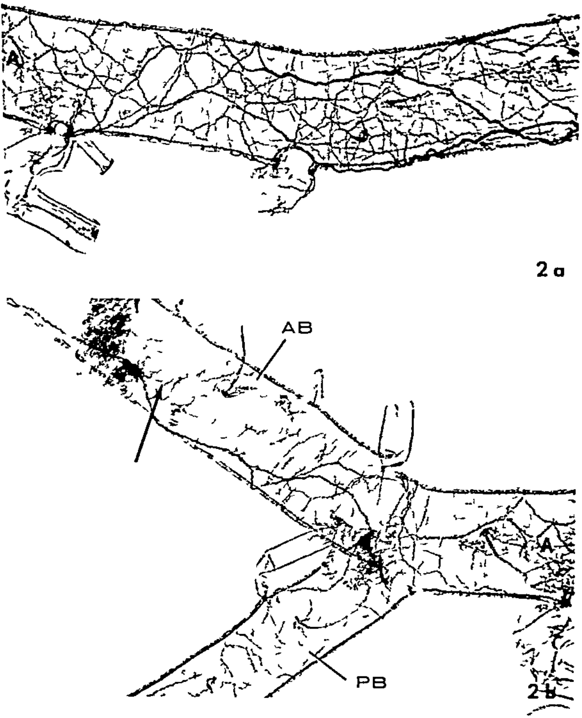
materials were processed as described ow The temporal bones of 5 experimental animals were decalcified in EDTA (disodium ethylenediaminetetraacetate) solution buffered with phosphate pH 7 1 (Ishii et al 1967) at 4 C for a week. Then the tissue blocks were frozen with dry ice and sectioned in a cryostat vertical to the long axis of the internal auditory *meatus at 12 μ After thawing and drying at* room temperature the sections were incubated in the substrate solution for the demonstration of AChE at 37°C for 90 min Finally the sections were rinsed with distilled water and mounted with PVP solution

To determine AChE iso-OMPA (tetra isopropylpyrophosphoramide in a final concentration of 10^{-4} M) was added to the incubating medium Solution without acetylthiocholine iodide was used as the medium in the control experiments

RESULTS

As described by Karnovsky & Roots (1964) copper ferrocyanide was deposited as light

Fig 2 AChE positive nerve bundles of the anterior inferior cerebellar artery × 53 (*a*) Proximal portion of the anterior inferior cerebellar artery Shortly after leaving the basilar artery (right side) the cerebellar artery is densely innervated by nerve bundles with AChE activity (*b*) Distal portion of the cerebellar artery directly continued from *A* The perivascular plexus with AChE activity becomes loosely organized towards the periphery The arrow points to where the artery crosses the orifice of the internal auditory meatus *AB* anterior branch *PB* posterior branch



2a

2b

lying in the loose connective tissue of the perivascular space in the bony labyrinth This diffuse AChE activity was also seen throughout the vascular wall of the stria vascularis and spiral ligament behind the external sulcus Neural structures however were not discerned on these blood vessels No enzyme activity was observed on the vessels of other areas in the inner ear

DISCUSSION

The presence of acetylcholine or choline acetyltransferase in the nerve ending suggests that the neuron is cholinergic but there are no convincing histochemical procedures for their demonstration AChE is widely distributed in nerves particularly cholinergic ones and is present in dendrites cell bodies axons and at the surface of nerve terminals (Potter, 1970) It is also known that the content of acetylcholine shows a good correlation with choline acetyltransferase and AChE activities in the peripheral nerves (Feldberg & Vogt 1948) Thus AChE activity in a neuron suggests a histochemical evidence of the cholinergic nerve fibers (Adams 1965)

The perivascular nerve plexus of the cerebral arteries consists of the sympathetic parasympathetic and sensory or afferent fibers Wasano (1968) demonstrated the adrenergic and cholinergic fibers of the cerebral and basilar arteries of rats using fluorescent and histochemical techniques He reported that both nerve fibers were distributed densely around the main trunks of the arteries on the lower surface of the brain, but that these fibers vanished in the peripheral portion of the vessels on the brain surface These observations were confirmed by electron microscopic studies demonstrating myelinated and unmyelinated perivascular nerve fibers around the arteries of the brain (Dahl & Nelson 1964 Sato 1966)

In the present study dense AChE active neural networks were observed around the basilar and anterior inferior cerebellar arteries but the latter rapidly lost its perivascular nerve towards the orifice of the internal auditory meatus This perivascular nerve plexus was seen to continue on the labyrinthine and common cochlear arteries But, this plexus was not observed under the light microscope in the peripheral cochlear and vestibular arteries

The adrenergic innervation of the arteries from the basal brain to the inner ear was studied by fluorescent microscopy Terayama et al (1966) observed the adrenergic perivascular nerve fibers in the cochlear branch of the modiolus but could not find them around the radiating arterioles and the vessels of the stria vascularis Spoendlin & Lichtensteiger (1966 1967) demonstrated a rich adrenergic nerve network in the basilar and labyrinthine arteries, but they observed no adrenergic fibers around the vessels of the stria vascularis

The cholinergic perivascular fibers as well as the adrenergic fibers were distributed only in the basilar, cerebellar and labyrinthine arteries The peripheral cochlear and vestibular arteries may not be under the influence of direct neurogenic control Thus the more peripheral portion than the labyrinthine artery may not demonstrate the same as do arteries elsewhere in the body

There is conflicting evidence in the literature concerning cholinergic modulation of inner ear blood flow (Nomura 1961 Morimitsu et al 1965 Suga & Snow 1969) The perivascular innervation patterns of the basilar artery markedly differ from that of vessels in other organs i e the small arteries and arterioles are usually innervated more densely than the large ones Thus concerning brain circulation only the main trunks and the larger arteries are under control of neural mechanisms

The inner ear seems to show similar characteristics The smaller arteries of the labyrinth do not demonstrate perivascular nerves whereas a sparse innervation was found around the labyrinthine artery continuing from that of the cerebellar artery This is merely a peripheral portion of the nerve plexus of the basilar artery The inner ear blood flow is possibly influenced by the basilar arterial system and also by the systemic blood circulation indirectly The

author s evidence is suggestive of the possibility that no independent neural regulation occurs in the arteries of the labyrinth

ZUSAMMENFASSUNG

Es wurde die Aktivitat der Acetylcholinesterase (AChE) im perivaskularen Nervenplexus der A basialis und A labyrinthi von Katzen histochemisch demonstriert Die A basialis wurde mit den dichten AChE aktiven Nervenbundeln und fasern umschlungen Die periarteriaren Nervennetze wurden in der A cerebellaris inferior anterior und A labyrinthi kontinuierlich beobachtet, aber AChE aktiven Nervenfasern waren in den peripharen cochlearen und vestibularen Arteri n lichtmikroskopisch nicht feststellen Obgleich die ganzen Gefasswande der Stria vascularis und Ligamentia spiralis eine AchE Aktivitat aufweisen wurden keine Nervenstrukturen konfirmiert. Da die perivaskulare AChE aktive Innervation bis zu der A labyrinthi und A cochleae communis verfolgt wurde konnte die neurogene Regulierung auf die Blutgefasse im Innenohr vom morphologischen Standpunkt aus-offenbar nur in diesem Gebiet beschrankt sein

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TYPES OF NEURONAL ACTIVITY IN THE LATERAL VESTIBULAR NUCLEUS

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Abstract A study of the responses of 150 single neurons in the lateral vestibular nucleus to a constant horizontal angular acceleration and deceleration of 4 /sec² for 50 sec revealed that 44 % of those found did not respond to stimulation These were termed type V neurons and may repre ent projections onto the lateral vestibular nucleus from the otolith organs from the spinal cord from unstimulated semicircular ducts or from some non vestibular system Type I (30 7 %) and type II (20 7 %) neurons in the lateral *vestibular nucleus appear to be the third-order* neurons which are activated by deflection of the cupula and connected with receptors in the semi circular ducts via internuncial second-order neurons connecting the vestibular nuclei or neurons relaying through the cerebellum There are fewer type III type IV neurons (those likely related to sensory) in the lateral vestibular nucleus than in medial vestibular nucleus The average resting frequency of type V neurons is higher than that of other types of neurons and the average resting frequency of neurons in the lateral vestibular nucleus is also higher than in the medial vestibular nucleus

The anatomical structure organization and location of the lateral vestibular nucleus (LVN nucleus of Deiters) and its connection to the central nervous system have been the object of numerous studies The LVN which is characterized by the presence of large multipolar cells, is well described by Brodal et al (1962) The primary vestibular afferents to the LVN (Gacek 1969) the spinal projections of the LVN (Pompeiano & Brodal 1957 *a*) the cerebello vestibular pathway to the LVN (Walberg et al , 1962) and other fiber pathways to the LVN have been studied extensively However, the gain in knowledge in the neurophysiology and information processing functions of the LVN has been slow In a previous study (Ryu et al 1969) the responses of 128 single neurons in the medial vestibular nucleus to caloric stimulations which has revealed some basic knowledge of the vestibular nuclei activity have been studied In the present study we have investigated the effect of the rotatory stimulation of the lateral semicircular ducts on the activity *of single neurons in the LVN*

This study was supported by grants NS-06785 from the National Institute of Neurological Diseases and Stroke National Institutes of Health

The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care

METHOD

Thirty one adult cats weighing 2 5 to 3 0 kg were used in this study The animal was anesthetized with an intraperitoneal injection of 35 mg/kg pentobarbital sodium the trachea was cannulated for the purpose of controlling respiration and the cat was fixed in a stereotaxic apparatus The occipital poles of the cerebrum and the anterosuperior part of the cerebellum were exposed through a 20 mm parieto occipital trephine and the posterior two-thirds of the bony tentorium was removed We drove a tung

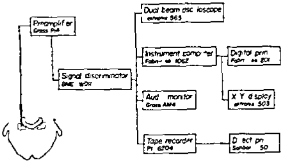


Fig 1 A block diagram showing the recording system

Types	Responses to rotatory stimulation		No. of neurons	Percent	Average frequency spikes per second
	acceleration	deceleration			
I	↓	↑	6	307 %	37 sps
I	↑	↓	3	207 %	33 sps
	↓	↓	2	13 %	29 sps
IV	↑	↑	5	33 %	24 sps
	NC		66	440 %	5 sps
Total			50	1000 %	39 sps

Fig 3 Classification and distribution of neurons in the lateral vestibular nucleus according to their responses to rotational stimulation *Arrow up* means an increase in frequency of action potentials occurred *arrow down* decrease *N C* no change

sten microelectrode (tip diameter 1 to 2 micra impedance 0 5 to 2 0 megohm) with a micro manipulator through the intact cerebellum into the LVN area of the left side of the brain stem stereotaxically using coordinates from stereotaxic atlas (Berman 1968 Snider & Niemer 1961) but depending heavily on Brodal s cytoarchitectural studies of the vestibular nuclei (Brodal et al 1962)

The microelectrode was connected (Fig 1) via a preamplifier and a signal discriminator to an instrument computer (Fabri tek 1062) for data analysis to one beam of a dual beam oscilloscope for direct observation and to one channel of an instrument tape recorder (Precision Instrument 6204) for data storage Audio monitoring was employed routinely and the frequency of the action potentials (spikes) during the resting state and during and after stimulation was displayed on the X–Y oscilloscope and printed out on the Kodak linagraph paper

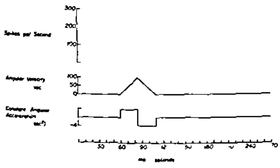


Fig 2 Profile of angular acceleration and velocity used in the experiment and concurrent frequency of discharges of a unit in the lateral vestibular nucleus

When single neurons in the lateral vestibular nucleus were found resting activity was recorded (Fig 2) a period of 60 sec and then the animal was subjected to a vestibular stimulus for typing The stimulus was a constant horizontal clockwise angular acceleration of 4°/sec for 25 seconds followed by an equal and opposite deceleration. The initial and final velocities were zero with a peak velocity of 100°/sec at the end of the acceleration phase

RESULTS

The effects of rotational stimulation on a total of 150 units in the lateral vestibular nucleus were studied All neurons studied were spontaneously active and some variation in resting frequency was noted from unit to unit in the same preparation firing rates ranged from 2 spikes per second (sps) to 124 sps with overall average resting frequency of 39 sps All of the neurons studied were found in the LVN area of the left side of the brain stem and were classified according to their responses (Fig 3) This classification is modeled after that of Duensing & Schaefer (1958) who using angular accelerations classified vestibular neurons according to their responses to ampullopetal and ampullofugal flow of endolymph Type I (Crampton s Ewaldian" unit) is an ipsilateral neuron that shows increased response when the cupula of the same side deviates to ampullopetal deflection and decreased response to ampullofugal deflection. Type II (Crampton s "non Ewal

dian unit) neurons show their response opposite to type I While type III neurons show decreased response to either direction of cupula deflection type IV neurons show increased response to either direction of cupula deflection And finally, type V neurons show no change in their response to both directions of cupula deflection As shown in Fig 3 the distribution of neurons according to their type, 46 (30 7%) out of a total of 150 neurons were type I 31 (20 7%) were type II 2 (1 3%) were type III 5 (3 3%) were type IV and 66 (44 0%) were type V The average resting frequencies according to their type were 37 sps for type I 33 for type II 29 for type III 24 for type IV, and 45 for type V

DISCUSSION

According to Lorente de No (1933 *a*) the utricular macula appears to be the main source of vestibular afferents to the lateral vestibular nucleus This study was supported by Stein & Carpenter (1967) and Gacek (1969) with their studies of the central projections of first order neurons supplying vestibular endorgan, and they also concluded that only the utricular afferents terminated on cells in the very small ventral portion of the LVN It is also a well recognized fact that the vestibulospinal tract derived exclusively from cells of the LVN is the principal pathway between the vestibular nuclei and spinal cord (Pompeiano & Brodal 1957 *b*) Based on those anatomical findings most of neurons in the LVN should belong to the type V group (which show no alteration of resting frequency on rotatory stimulation) and we expected to see very few number of units of other types We did find however a significant number of type I and type II neurons and a few type III and type IV neurons

Since sixty six (44 0%) of neurons were found to be type V and this was the largest group in the LVN these may represent projections onto the lateral vestibular nucleus from the otolith organs Many of these neurons could also represent projections from the vestibulospinal tract, from unstimulated semi circular ducts, or from some non vestibular system

Shimazu & Precht (1965) suggested that type I and type II neurons are the second order neurons in the vestibular nuclei which are primarily connected with receptors in the semi circular ducts and activated by deflection However type I and type II neurons in the LVN are not likely directly connected to the primary fibers, because Stein & Carpenter (1967) and Gacek (1969) did not find any degenerated fiber endings in LVN after producing electrolytic lesions in the canal nerves of the vestibular ganglia Considering this finding the type I and type II neurons we recorded in the LVN are likely the third order neurons Anatomic evidence (Brodal et al , 1962) for this is that there are within the medial and descending vestibular nuclei cells with short axons passing to and ending in the lateral vestibular nucleus i e true internuncials The other possible explanation is that LVN type I and II neurons are stimulated by primary fibers relaying through the cerebellum (Stein & Carpenter 1967)

Duensing & Schaefer (1967) suggest that both type III and type IV neurons are functionally related to the reticular activating system The type III neuron (which decreases in activity after vestibular stimulation) may be innervated directly from each endorgan without an intercalated inhibitory neuron or its response may be mediated through another non vestibular sensory neuron The latter supports anatomic evidence that sensory impulses other than vestibular may reach the vestibular nuclei (Pompeiano & Brodal 1957 *a*) Type IV neurons exhibited an increase in activity after vestibular stimulation pinching the ears or merely pulling whiskers The number of these type III and type IV neurons appear to be scanty in the LVN as compared with the medial vestibular nucleus (Ryu et al 1969) Lorente de No (1933 *b*) and Scheibel & Scheibel (1958) stated that the medial two-thirds of the pontomedullary reticular formation has synaptic connec

tions with the vestibular nuclei The sum of evidence is very strong that type IV neurons are related to the reticular activating system

The resting frequency of type V neurons is higher than the resting frequency of type I and II For this reason we suspect that the type V neurons are likely to receive input whose spontaneous activity is higher than the spontaneous activity of primary vestibular neurons The average resting frequency of neurons in the lateral vestibular nucleus (about 39 spikes per second) is higher than the average resting frequency of neurons in the medial vestibular nucleus (about 23 spikes per second) This result is reasonable considering that LVN contains the considerable number of type V neuron and MVN contains large number of type I and II and very few type V neurons

ZUSAMMENFASSUNG

An 150 Neuroneneinheiten in dem lateralen Vestibulariskern wurde die Antwort auf konstante 50 sek lang wahrende horizontale Winkelbeschleunigung und verzogerung von 4 /sek untersucht Es ergab sich dabei, dass 44% der Neuronen keine Reaktion auf die Stimulation zeigten Diese Neuronen wurden als Typ V bezeichnet und konnen Projektionen in den lateralen Vestibulariskern von den Otolithorganen vom Ruckenmark von den nichterregten Bogengangen oder von einem nicht zum Vestibularapparat gehorenden System sein Bei den Neuronen vom Typ I und Typ II die sich zu 30 7% bzw 20 7% im lateralen Vestibulariskern fanden handelt es sich anscheinend um Neuronen 3 Ordnung die durch Deflexion der cupula aktiviert werden und *zu denen von Rezeptoren in den* Bogengängen her uber Zwischenneuronen 2 Ordnung die die Vestibulariskerne miteinander verbinden oder uber Schaltneuronen im Kleinhirn verlaufende Bahnen fuhren Es zeigte sich weiter dass die Zahl der Neuronen vom Typ III und Typ IV die wahrscheinlich mit der sensorischen Integration in Beziehung stehen im lateralen Vestibulariskern geringer ist als im medialen Vestibulariskern Schliesslich konnte festgestellt werden dass die durchschnittliche Ruhefrequenz der Neuronen vom Typ V hoher ist als die der Neuronen anderen Typs und dass die Neuronen im lateralen Vestibulariskern eine hohere Durchschnittsruhefrequenz als die im medialen Vestibulariskern haben

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MATHEMATICAL MODELLING

A Contribution to Clinical Vestibular Analysis

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Abstract A model of the vestibulo-ocular system used by the authors to explain mathematically some peculiarities of the nystagmus patterns and general phenomena such as vestibular adaptation and secondary nystagmus is illustrated after a brief revue of the current status of vestibular modelling The model has been implemented on a digital computer and several rotational tests have been simulated By comparing the experimental data obtained from young healthy subjects with the model predictions it has been possible to define the model parameters quantitatively The results of these investigations are reported in this paper Finally a method for an automatic analysis of the vestibular function from E N G data recorded during post rotational tests is discussed in the concluding section

The electrical registration of ocular deviation by electronystagmography (E N G) has assumed the status of a preferred almost exclusive procedure for the objective examination of vestibular function in many clinical and research situations The elements usually considered in E N G tracings are nystagmus duration frequency and number of nystagmus beats total angular displacement or *Gesampt amplitude* and slow phase angular velocity (Aschan et al 1956 Megighian 1959)

At the moment there is a good deal of controversy among the clinicians about which of these E N G elements is the most expressive More realistically many authors point out on all these elements Some electronic and electromechanical devices have been recently introduced for a direct measurement of a number of these elements (Benson & Stuart 1967 Guedry & Turnipseed 1968 Voots 1969) Digital computers have been also used for an automatic analysis of the E N G tracings (Johnson et al 1967 Herberts et al 1968 Torok & Derbyshire 1968)

Following early suggestions by the Utrecht School (van Egmond et al 1949) several investigators are used to derive nystagmus cupulograms from slow phase eye velocity in post rotational nystagmus mainly for purpose of research This allows evaluating the cupula restoring time constant According to Steinhausen s equation (Steinhausen 1931) likening the cupula endolymph system to an over damped torsion pendulum this time constant represents the ratio between the viscous torque per unit relative angular velocity of endolymph and the moment of cupula restoration force per unit angular displacement of endolymph

The assumption that slow phase eye angular velocity is proportional to cupula deflection has been proved to represent a good approximation for the analysis of the dynamical behaviour of the cupula endolymph system Therefore this approach may be advantage

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ously followed also for clinical diagnosis of vestibular disorders of peripheral origin On the other hand the clinical experience proves that only few vestibular diseases are due to alterations of the physical characteristics of the cupula endolymph system If the same ap proach has to be followed for clinical investiga tions of central vestibular disorders the model describing the dynamics of the neural pro cesses in the vestibulo ocular reflex by means of a simple integrator becomes completely un satisfactory

An attempt to give a better mathematical description of the vestibulo ocular system has been made in the last years by several in vestigators at the Massachusetts Institute of Technology (Meiry 1965 Steer 1967 Young 1968 Young & Oman 1968) and at the McGill University (Sugie & Jones 1965 Outerbridge 1969 Malcolm & Jones 1970)

Some authors tried to explain the pattern of the nystagmus beats by means of models which on the other hand fail in predicting general phenomena such as vestibular adapta tion and secondary nystagmus (Sugie & Jones 1965 Outerbridge 1969)

Some other authors have made an attempt to improve the second order torsion pendulum model for the semicircular canals by including a mathematical descriptor for the effects of short term adaptation (Young & Oman 1968 Malcolm & Jones 1970) However they did not suggest any model for the neural mechan ism which produces the nystagmus beats

Very recently a mathematical model by Schmid (1970) and by Schmid & Stefanelli (1970) seems to predict some peculiarities of the nystagmus patterns and to explain vestibu lar adaptation and secondary nystagmus In previous works these authors have carried out a mathematical analysis of the model No particular attempt has been made at that time to define quantitatively the model parameters Subsequently the model has been implemented on a digital computer and several vestibular rotational tests have been simulated The re sults have been compared with the E N G

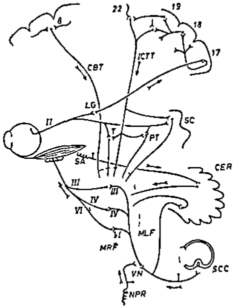


Fig 1 A schematic of the major known elements of the oculomotor system *CBT* corticobular tract *CER* cerebellum *ICTT* internal corticotectal tract *LG* lateral geniculate body *MLF* medial longitudinal fasciculus *MRF* mesencephalic and pontine reticular formations *PT* pretectal nuclei *SA* stretch afferents from extraocular muscles *SC* superior colliculi *SCC* semicircular canals *T* tegmental nuclei *VN* vesti bular nuclei *II* optic nerve *III IV* and *VI* oculomotor trochlear and abducens nuclei and nerves *17 18 19* and *22* primary and association visual areas occipital and parietal *8* frontal eye fields *NPR* neck proprioceptive receptors (Modified from Robinson 1968)

records obtained by performing the same tests on healthy human subjects In such a way it has been possible to confirm the validity of the model and to specify the model parameters quantitatively

The purpose of this paper is to present the results of the latter investigations A brief description of the model is first given for a better understanding of the results which fol low In the concluding section a method for an automatic analysis of the vestibular func tion from E N G data recorded during post rotational tests is discussed

The model

It is well known (Jones & Milsum 1965 Robinson 1968 Kornhuber 1969) that eye movements arise from the action of a multi

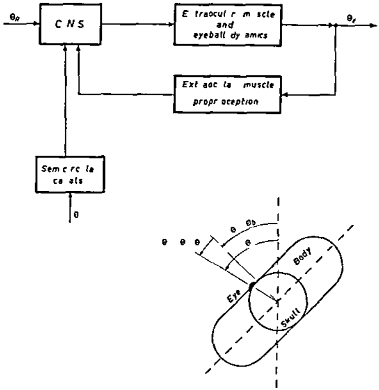


Fig 2 Simplified information flow diagram of the vestibulo-ocular system θ skull angular position θ body angular position θ eye angular position θ eye in skull angular position θ reference signal

loop control system which contains four main feedback pathways visual vestibular and two proprioceptive (eyes in skull, skull on body) A schematic of the major known elements of the oculomotor system is given in Fig 1

If vestibularly induced eye movements dur normal rotational tests are considered only a part of the system shown in Fig 1 becomes of interest Namely assuming the subject sits on a chair rotating about a vertical axis in total darkness with the body and skull fixed to the chair the visual and neck proprioceptive feedbacks are excluded Area 8 is also excluded if the subject is recommended not to produce voluntary eye movements A block diagram of the simplified system is shown in Fig 2

The role of the extraocular muscle proprioceptors has been discussed by many authors (Whitteridge 1960 Robinson 1968 Fuchs & Kornhuber 1969 Matyushkin & Aver Yanova 1969) Although the projection of the extraocular muscle afferents to the cerebellum has been recently proved (Fuchs & Kornhuber 1969) at the moment no one knows with any certainty what use the central nervous system makes of the stretch afferent signals Anyway there is a good deal of experimental data which tend to support the hypothesis that extraocular muscle proprioceptors do not play an essential role in vestibularly driven eye movements (Whitteridge 1960 Matyushkin & Aver Yanova 1969)

In attempting to give a mathematical description of the vestibulo-ocular system behaviour it has been assumed that a signal proportional to eye relative position is available at the level of the C N S (Schmid 1970 Schmid & Stefanelli 1970) It was beyond the purpose of that research and is so even in this paper to establish whether this signal is given by the extraocular muscle proprioceptors or by a central mechanism which receives information from the vestibular system

The final model of the vestibulo ocular system proposed by Schmid and Stefanelli is represented in Fig 3 This model intends to predict eye movements produced by stimuli of

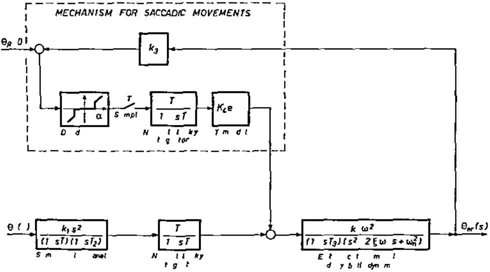


Fig 3 The model of the vestibulo-ocular system T and T short and long time constants of the semi circular canals T adaptation time constant T time constant of the saccadic neural leaky integrator T and ω time constant, damping factor and natural frequency of the extraocular muscles and eyeball α threshold value of the d ad zone T sampling period τ time delay k k k k_s, and k gain factors s Laplace complex variable

the lateral semicircular canals under the par ticular test conditions specified above

The dynamics of the various blocks of the diagram in Fig 3 is now briefly illustrated

The transfer function of the pair of lateral semicircular canals corresponds to Stein hausen s equation (Steinhausen 1931) and the time constant T_1 and T are given by (van Eg mond et al 1949 Jones & Spells 1963 Mayne 1966)

$$T_1 \simeq \frac{I}{\tau} = \frac{4\eta}{or} \quad \text{(short time constant)}$$

$$T \simeq \frac{\tau}{\Delta} = \frac{8\tau\eta R}{\mu r^2} \quad \text{(long time constant)}$$

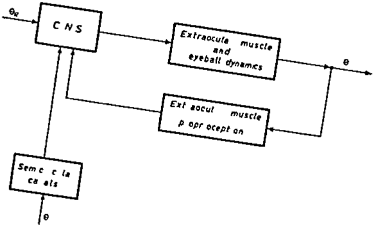
where

- I = moment of inertia of the endolymph
- τ – viscous torque per unit relative angular velocity of the endolymph
- Δ – moment of the cupula restoration force per unit relative angular displacement of the endolymph
- r – internal radius of canals
- R – radius of canals
- η = coefficient of viscosity of the endolymph
- o = density of the endolymph
- μ = coefficient of elasticity of the cupula

The transfer function describing the dyna mics of the extraocular muscles and eyeball can be derived from Thomas (1967) or from Robinson (1968) The transfer functions proposed by these authors are not too different In the block diagram of Fig 3 Thomas transfer function has been assumed to relate muscle torque to eye angular relative position The values proposed by Thomas for the transfer function parameters are

K_0 = 0 291 deg/g
ω = 380 rad/sec
ξ = 0 51
T_3 = 0 0368 sec

The neural leaky integrator in the primary pathway (lower part of the diagram in Fig 3) has been introduced to obtain eye movements which compensate head movements In fact it has been proved by many authors (Jones & Milsum 1965 Mayne 1966) that cupula de-



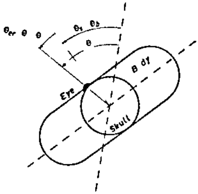


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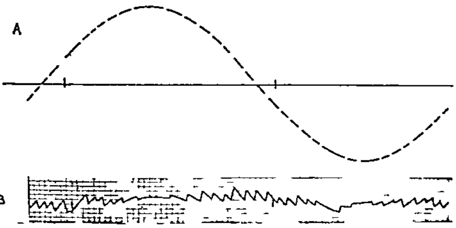


Fig 5 Cumulative eye position (*A*) corresponding to the horizontal eye nystagmus (*B*) produced by a sinusoidal acceleration of 40 /sec² and 0 07 cps

Evaluation of the model parameters via clinical vestibular analysis and computer simulation

According to control theory linear time in variant dynamical systems can be identified by considering their frequency response Frequency response analysis consists in forcing the system with a sinusoidal input of variable frequency and measuring the amplitude ratio and the phase shift between the system output and in put If the amplitude ratio and the phase shift are plotted against the frequency the frequency response diagrams are obtained

When the semicircular canals are stimulated sinusoidally the induced eye movements present the nystagmoid waveform shown in the record ings of Fig 4 By eliminating the nystagmus fast phase and fitting together the smooth por tions of the slow phase a diagram is obtained which represents the cumulative eye position (Meiry 1965)

As shown in Fig 5 the cumulative eye posi tion in response to a sinusoidal stimulus varies sinusoidally Therefore the frequency response diagram of the vestibulo-ocular system can be constructed considering the head angular posi tion as system input and the cumulative eye position as system output

The frequency domain analysis carried out by Hixson & Niven (1962) and by Meiry (1965) has provided the experimental data reported in Fig 6

A simple analysis of the model in Fig 3 shows that if $T > T$ the leak of the saccadic integrator has a very little influence on the model response to sinusoidal inputs in the range of frequency which can be of interest On the other hand if the saccadic leaky integrator is treated as a perfect integrator the cumulative eye position corresponds by definition to the model output when the saccadic pathway is supposed to be opened Therefore the predicted cumulative eye position θ is related to the input θ (head angular position) by the following transfer function

$$G(s) = \frac{\theta_{ec}(s)}{\theta(s)} = \frac{-KT\omega s^2}{(1+sT_1)(1+sT_2)(1+sT)(1+sT_3)(s^2+2\xi\omega s+\omega^2)} \quad (1)$$

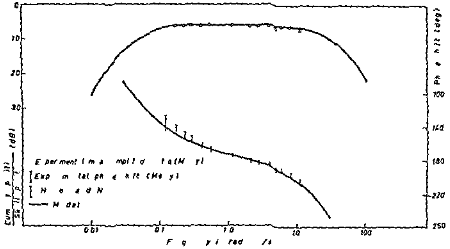


Fig 6 Frequency response of the vestibulo-ocular system

where

$K = K_0 K_1$

By letting $s = j\omega$ into (1) the diagrams of the predicted frequency response can be obtained Namely by definition of transfer function it is

$$|G(j\omega)| = \text{amplitude ratio} \quad (2)$$

and

$$\measuredangle G(j\omega) = \text{phase shift} \quad (3)$$

between the predicted cumulative eye position and the head angular position for sinusoidal rotation of frequency ω

Assuming for ω_n ξ and T_3 the values proposed by Thomas (1967) it is possible to evaluate K T_1 T and T_a from the experimental data plotted in Fig 6

Due to the lack of data relative to the system response to high frequency sinusoidal inputs only a rough estimation of the short time constant T_1 is possible The solid lines in Fig. 6 represent the frequency response diagrams computed from (2) and (3) assuming $K = 9$ $T_1 = 0.01$ sec $T_2 = 18$ sec and $T_a = 60$ sec

It is worth noting that the values of the canals short and long time constants computed in this way are in good agreement with the experimental results obtained by van Egmond et al (1949)

As far as the saccadic pathway is concerned it can be reasonably assumed that the same mechanism operates to generate saccades either in eye tracking movement and in vestibular nystagmus Therefore we can attribute to T and τ the values computed by Young (1962) for the refractory period and the reaction time of saccades in eye tracking movement

Then

$T = 200$–220 msec

$\tau = 160$–180 msec

The parameters K_3 and α cannot be estimated separately Thus we can assume $K_3 = 1$, and derive the value of α from an analysis of the amplitude of the nystagmus beats and their number in a period of the stimulus during sinusoidal rotation tests A good agreement with the experimental data has been obtained by taking

$\alpha = 6$–$8°$

The value of K_4 has been fixed by letting

$K_4 = 1/K_0$

In such a way the saccadic jerks would compensate exactly the eye displacement during the slow phase if the saccadic delay did not exist

Using the values of the model parameters so far determined and neglecting the leak of the



Fig 7 Predicted nystagmus for the sinusoidal rotational test which gave the last record in Fig 4

saccadic integrator the sinusoidal rotational test which gave the last recording in Fig 4 has been simulated and the result is shown in Fig 7 The fast components of the predicted nystagmus follow vertical lines since the dynamics of the extraocular muscles and eyeball has been neglected for sake of simplicity

The last model parameter to be determined is the time constant T of the leaky integrator in the saccadic pathway It has been shown by Schmid (1970) that the main effect related to the leak of the saccadic integrator is the difference between the total eye displacement during the first and the second phase of postrotational nystagmus Therefore we carried out postrotational tests and tried to determine T_s by fitting the cumulative eye position predicted by the model with that reconstructed from the experimental data. The results of this analysis have proved T generally exceeding 150 sec

In Fig 8 the cumulative eye position constructed from a 180 /sec postrotational nystagmus is shown together with the cumulative model output computed for the following values of the model main parameters

K = 11 35

T = 18 2 sec

T = 61 1 sec

T = 155 6 sec

To confirm the validity of the proposed model higher order input have been considered In particular a rotational test has been carried out by programming the chair angular velocity as shown in Fig 9 A first ramp of velocity has been generated by an angular acceleration of 2 7 /sec lasting until the angular velocity of 240 /sec is reached This velocity has been maintained for the following 100 sec Then a second ramp velocity in the opposite direction has been produced by a deceleration of 1 4 /sec[1] When the chair velocity went

All the experimental data reported in this paper have been obtained from rotational tests carried out in total darkness Subjects had the head secured in a holder and inclined 30 forward A Beckman Dynograph Type R with a 3 sec time constant was used in amplifying and recording the eye movement signals All the subjects examined were healthy young students

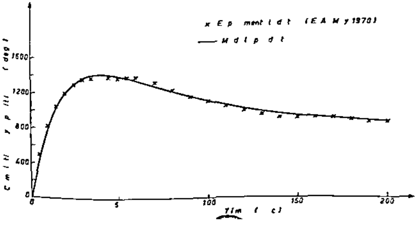


Fig 8 Cumulative eye position from a 180 /sec postrotational n

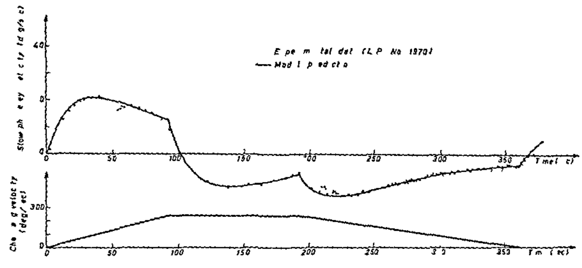


Fig 9 Model prediction for a higher order input

through zero the chair was automatically blocked

Dots in Fig 9 show the nystagmus slow phase velocity plotted as a function of the time after onset of the stimulus The solid line in the same figure gives the results of the computer simulation for the following values of the model main parameters

$K = 12\ 87$

$T = 20$ sec

$= 70$ sec

$T_s = 1\ 000$ sec

The close match between model response and experimental data strongly supports the validity of the model

Concluding remarks and future developments

The analysis carried out in the preceding section suggests the opportunity of distinguishing the model parameters into two different sets

The first set contains the parameters which determine the shape of the cumulative eye position in vestibularly induced nystagmus i e (*a*) the partial gain K of the smooth pathway (*b*) the long time constant T of the semicircular canals, (*c*) the adaptation time constant T (*d*) the time constant T_s of the saccadic leaky integrator

As previously outlined the canals short time constant T_1 as well as the extraocular muscles and eyeball dynamics have little influence on the overall system behaviour

The second set contains the parameters which for given values of the preceding ones determine the pattern of the nystagmus tracings i e (i) the time delay τ, (ii) the sampling period T (iii) the threshold a for the saccadic signals

The model parameters of the first set can be conveniently estimated from the diagram of the cumulative eye position constructed from the recording of postrotational nystagmus The time constant T_s which remains practically masked in sinusoidal responses can be easily computed if the postrotational nystagmus is recorded for a time sufficiently long (4–5 min)

Fig 10 shows the way we are following for an automatic elaboration of the E N G tracings and the estimation of K T_1 T_a and T_s A postrotational test is carried out, and the induced nystagmus is recorded and converted into digital data The cumulative eye position is then computed using a special program appointed for this purpose The postrotational test is also simulated on the digital computer by forcing the model with a proper step velocity input The cumulative eye position predicted by the model is compared with the cumulative eye position obtained from the ex

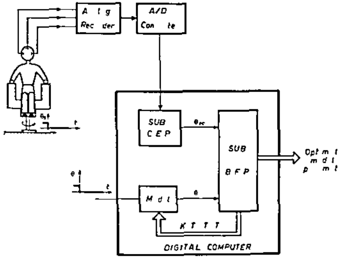


Fig 10 Automatic elaboration of the ENG records θ chair and skull angular velocity θ cumulative eye position θ cumulative eye position predicted by the model for given values of K T T and T SUB CEP subroutine for the construction of the cumulative eye position SUB BFP subroutine for a best fitting procedure

perimental data A best fitting sub program varies the model parameters K T_s T and T until an optimal matching is obtained

By means of such an automatic method of analysis it is possible to derive the mean values of the parameters of the first set either for normal subjects and for subjects with classical vestibular disorders The expected result will be the construction of a number of regions in the four dimensional space of the parameters K T T and T Each region will correspond to a particular status of the vestibulo-ocular system

A large program has been undertaken in this direction Obviously the first point to be proved is that parameters variations due to certain vestibular disorders are large enough to be appreciated within the accuracy range of the method followed for parameters estimation

It can be expected that some diseases will not cause marked variations in cumulative eye position though they will mainly influence the nystagmus pattern Therefore the same kind of analysis should be carried out also in the space of the parameters τ T and α of the second set

The automatic diagnosis of several vestibular disorders is the goal of the research

ACKNOWLEDGMENTS

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ZUSAMMENFASSUNG

Nach einem kurzen Bericht der gegenwartigen vestibularen Modellierung wird hier ein vestibulo-ocularis Modell beschrieben um einige Eigenschaften der Nystagmustypen und allgemeine Phanomene (vestibularis Adaptation sekundaren Nystagmus) mathematisch zu erklaren Das Modell wird auf einem Digitalcomputer aufgestellt und verschiedene Rotationsteste werden ausgefuhrt Durch Vergleichung der bei jungen gesunden getesteten Leuten erhaltenen Ergebnisse mit Modell Resultaten ist es moglich die Modellparameter zu bestimmen die Ergebnisse dieser Nachforschungen werden in dieser Schrift gegeben Zum Schluss wird eine Methode zur Analyse der vestibularis Funktion durch ENG Ableitung bei postrotatorischen Prüfungen besprochen

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VESTIBULAR PHYSIOLOGY IN A STRAIN OF THE WALTZING GUINEA PIG

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Abstract The vestibular physiology was studied in a strain of the waltzing guinea pig with a dominant mode of inheritance Rotation did not elicit any nystagmus response and tilting failed to evoke counter rolling of the eyes In young waltzers a caloric response could be elicited by combining an immediately preceding fenestration of a semicircular canal with irrigation of the bulla with hot and cold water No such reaction occurred in older animals All waltzers showed impaired performances in behavioural tests for swimming ability and righting reflex irrespective of age The galvanic response was normal at any age Scarpa s ganglion had a normal appearance when examined under the light microscope The conclusions drawn are that the waltzers of this strain are born with a decreased vestibular capacity and that this impairment is progressive presumably until total loss of function The results are in agreement with earlier reports on the vestibular morphology showing progressive neuroepithelial degeneration

Many reports have been published on inner ear physiology in animals with genetic deafness Interest has centered mainly however on cochlear physiology In rodents there are several mutants with inherited deafness that also display abnormal locomotion e g shaking or waltzing possibly initiated by vestibular dysfunction (cf Deol 1968)

The waltzing guinea pig was first described by Ibsen & Risty in 1929 Their strain showed a recessive mode of inheritance later confirmed by Ibsen (1932) and Lurie (1939 1940 1941) Peripheral vestibular reactions could not be elicited but light microscopy did not reveal degeneration in the vestibular end organs (Lurie 1939 1940 1941 Cogan 1940) An interruption of the reflex arc between the labyrinths and the eyes was postulated Guinea pigs that behaved normally showed a waltzing like type of locomotion after destruction in the brain stem (Lurie & Dempsey 1939 Buchanan 1940)

At the King Gustaf V Research Institute a strain of the waltzing guinea pig has been bred systematically the descendants of six waltzers received from the National Institutes of Health in 1961 Genetic analysis showed a dominant Mendelian mode of inheritance with a recessive lethal effect (Ernstson 1970) Morphologic studies on the vestibular end organs showed even at birth marked and with increasing age progressive degeneration of the neuroepithelium (Ernstson et al 1969 1970) A proteinoid rod shaped inclusion body was regularly observed in the hair cells type I

The present study was undertaken to elucidate the vestibular physiology in this specific strain of the waltzing guinea pig

MATERIAL

A total of 991 animals have been examined Even on superficial inspection the waltzing guinea pigs exhibited typical wal

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haviour random periods of circling in any direction Furthermore they had a peculiar habit of slightly tilting their heads upwards and sideways However, only to observe their behaviour was not sufficiently conclusive for ascertaining the waltzing character especially not during the immediate postnatal period

METHODS

Screening tests

The animals were rotated at least a full turn at moderate speed The presence of ocular and head nystagmus reactions were observed The guinea pigs were then tilted about 45° and examined for counter rolling of the eyes

Rotation test

The animals were put in a restraining cage equipped with a head holding device and placed on a rotation table Needle electrodes were inserted subcutaneously at the temporal canthus of each eye and in the midline of the nose To exclude visual stimuli a dark box was placed over the animals Conventional electronystagmographic recordings were made After a counterclockwise linear acceleration amounting to 14.5° sec^{2} the table was rotated t a constant speed of about 120°/sec for sec It was then brought to a standstill in s than a second The same procedure was repeated but in a clockwise direction after a suitable intermission

Caloric test

Fenestration of the left lateral semicircular canal was performed immediately preceding irrigation An incision was made under local anaesthesia above the auricle the bulla was exposed and widely opened above the ear canal In this way an adequate approach was obtained to the lateral and anterior semi circular canals The bony covering was re moved from the lateral canal near the ampule without rupturing the membranous semicircu lar canal.

The animals were then placed in a restraining cage equipped with a device that held the head in a normal position Needle electrodes were inserted as in the rotation test and E N G -recordings were made accordingly Irri gation of the fenestrated middle ears was per formed with warm (51°C) and cold (6–14°C) water each douching lasting about 60 sec

Behavioural tests

The ability to swim was observed The righting reflex was studied in the following manner The animals were allowed to fall about 40 cm in the dark starting with their feet upwards A very slight impact on a pressure sensitive pad triggered an electronic flash and a camera recorded the animals position Thus pictures were taken at a moment when the bodies were still in the air and the leading parts had but slightly touched the pad

Galvanic test

The animals were restrained by the neck in a simple device, and needle electrodes were in serted subcutaneously behind both ears The animals eyes were observed for deviation and nystagmus like responses while the tension between the electrodes was slowly increased from zero until a reaction occurred The threshold voltage for each response was noted

Light microscopy

Celloidin sections of temporal bones, processed as earlier described (Ernstson et al 1969) were examined under the light microscope

RESULTS

Screening tests

The screening tests served to establish the waltzing character confirming the impression gained from the behaviour of the animals The results were almost stereotypically uni form Thus 466 guinea pigs were shown to be waltzers on rotation they exhibited neither nystagmus nor head nystagmus and tilting did not elicit counter rolling From the 525 normal animals the anticipated responses to both tests were obtained Only in four cases was the

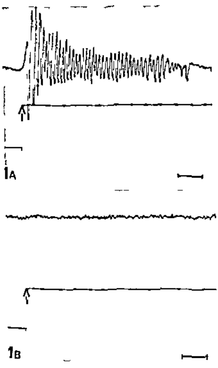


Fig 1 Rotation test (A) Postrotatory nystagmus in a normal guinea pig (368 1 / months old) (B) Absence of reaction in a waltzing guinea pig (29IV 1 month old) The arrows indicate the momen of sudden retardation Time bar 1 sec

immediate postnatal result not confirmed at a subsequent control

Rotation test

In this way 51 animals were examined 31 waltzers (age 1–155 days) and 20 normals (10–55 days) An acceleration of about 10 /sec was found necessary to elicit per rotatory nystagmus in the normal guinea pigs All the normals regularly showed nystagmus according to rule its duration however was seldom appreciably longer than the acceleration The sudden retardation elicited a post rotatory nystagmus of uniform duration in all the normal animals In the waltzing guinea pigs however neither per nor post rotatory nystagmus was elicited (Fig 1 A B)

Caloric test

Fifteen animals were examined five adult normal guinea pigs five waltzers less than 1 month old and five waltzers more than 1 ¹/ years of age The reaction of the normal guinea pigs to fenestration was inconspicuous and of short duration Nystagmus was not recorded when douching started All normals except one responded with a left beating nystagmus during irrigation with warm water A right beating persistent nystagmus was evoked in the exceptional animal Cold water irrigation caused nystagmus directed to the right and this persisted as a spontaneous nystagmus in four of the animals Further tests with warm water caused either a left beating nystagmus or a decrease in the right beating spontaneous nystagmus (Fig 2 *A*) After three irrigations with warm and cold water all the animals exhibited a persistent right beating nystagmus with a manifest change of behaviour head nystagmus to the right and the body inclined to the left

Three of the young waltzers showed a response to fenestration similar to that of the normals whereas two failed to react Three of them responded with a left beating nystag mus during the first warm water irrigation a persistent right beating nystagmus was evoked in one and in the fifth there was no reaction All of them however reacted with right beat



Fig 2 Caloric test last douching sequence (A) A normal guinea pig (713 22 months old) The animal showed a persistent right beating nystagmus after previous sequences Irrigation with water at 51 C (started at arrow) caused a change to left b ating nystagmus (B) An old waltzer (612\ 27 months old) No visible reaction to earlier irrigations and none to repeated douching with water at 51 C (started at arrow) Time bar 1 sec

Table I *The attitude of the animals on landing after falling about 40 cm in the dark from an upward down position The normal group and the waltzer group consisted of 19 and 20 animals respectively*

Attitude at landing	Normals (32 tests)	Waltzers (50 tests)
Head position		
Turned < 90	0	34
Turned > 90	32	16
Leg position		
0–1 leg downwards	0	32
2–3 legs downwards	14	17
4 legs downwards	18	1

ing nystagmus during the first test with cold water At subsequent douchings with warm water three showed adequate caloric reactions according to rule After three test sequences all the animals presented a persistent right beating nystagmus and a change in behaviour conforming to that of the normals

None of the older waltzers reacted visibly to fenestration No reaction to the first irriga tion with warm water was observed One of them the youngest showed a right beating nystagmus in response to cold water three acted with some irregular eye movements d the fifth did not react at all Uniform esults were obtained at repeated douchings (Fig 2 *B*) Persistent nystagmus was not seen in the old waltzers nor was any change in behaviour observed

Behavioural tests

Five normal animals 1–42 days of age and 50 waltzers 1–160 days old were observed when swimming The normal guinea pigs swam excellently keeping a straight course The waltz ing animals could also swim but kept a straight course for only a very short distance and then invariably started to swim in a circle ending in a steep downward spiral course under water and had to be rescued All the waltzers acted alike

The results of the righting reflex test are summarized in Table I Twenty waltzers, aged between 1 week and 6 months were each tested 2–3 times 50 tests in all A series of 32 tests were made on 19 normals of similar age distribution The guinea pigs endeavoured to turn in the air All normal guinea pigs turned their heads more than 90° nearly always a full turn of 180° whereas in 34 tests the waltzers turned their heads less than 90° The normals landed on all four feet in 18 tests out of 32 only once in 50 falls did a waltzer accomplish the same manoeuvre (Fig 3 *A B*)



3A



3B

Fig 3 Behavioural test falling in the dark The pictures show the position at landing (A) A normal guinea pig (B) A waltzing guinea pig (272\ 4 months old)

Galvanic test

The results are presented in Table II With this procedure 108 animals were studied 58 waltzing and 50 normal guinea pigs Both groups had a corresponding age distribution from 2 days to more than 1 year The thresh

Table II *Thresholds for eliciting galvanic eye movements*

Group	Galvanic tension (volts) to elicit			
	Eye deviation		Nystagmus-like reaction	
	p50	p25–75	p 0	p25 75
Normals $n=50$	1 6	0 3	2 5	0 4
Waltzers $n=58$	1 5	0 5	2 4	0 6

olds for galvanic reactions were the same in normal and in waltzing guinea pigs The responses were identical in both groups

Light microscopy

Scarpa s ganglion was examined in 50 waltzers and in 10 normals of different ages No abnormality was observed even in the very old waltzers

DISCUSSION

The results of the present investigation show a marked loss of elicitable vestibular function *even in the perinatal period The absence of* per and post rotatory nystagmus and the diminished righting reflex are in close agreement with previous studies on similar animals with recessive inheritance (Lurie 1939 1940 1941 Cogan 1940)

Angular acceleration is the adequate stimulus for the semicircular canals The normal guinea pigs however did not show recordable per rotatory nystagmus at accelerations less than about 10 /sec thus exhibiting a comparatively high threshold The upper limit for the available rotation table was 14 5 /sec Thus the suprathreshold stimulus for eliciting per rotatory nystagmus was not impressive The retardation exceeding 100 sec must be regarded as a more intense stimulus However neither a per nor a post rotatory nystagmus response was recorded in any waltzer irrespective of age

Normal guinea pigs reacted by falling into a catatonia like state— Totstellung —and showed no nystagmus reaction when conventional warm caloric irrigation was tried Eviatar & Goodhill (1968) were unable to elicit in rabbits nystagmus with warm calorization *even with the use of hot water at 60 C* for a 2 min irrigation period

These obstacles were surmounted by applying a more powerful stimulus by combining an immediately preceding fenestration of the semicircular canal with irrigation of the bulla with warm and cold water The nystagmus reaction to this combined procedure was as might be expected of two types The first irrigation always of the left ear and invariably applying warm water caused a left beating nystagmus almost without delay This response subsided soon after the stimulus Cold water irrigation then evoked a right beating nystagmus Though this became persistent at this stage or after subsequent douchings further warm water irrigations caused either a left beating nystagmus or a decrease in the right directed persistent nystagmus This reaction must be interpreted as a regular caloric response

The persistent right beating nystagmus that developed and the behaviour of the animals after completing the test procedure had the characteristics of a destructive peripheral vestibular lesion The irrigations were performed with pure water It is thus conceivable that this destructive reaction was in part caused by an osmolar gradient (Wittmaack 1956 Arslan 1969) It should be observed that the older waltzers did not respond in either way

Behavioural tests for the functional condition of the vestibular apparatus gave consistent results An impaired ability to swim has been used as a sign of vestibular insufficiency in several mutants of hereditary deaf mice (e g Quix 1907 Deol 1956 1968 Gruneberg 1956) Six of these mutants all of which could swim at an early age lost this proficiency with increasing age The waltzers of the present strain showed an impaired swimming ability even at the perinatal period No further de

terioration in this respect was observed with increasing age.

Earlier reports on waltzing guinea pigs and rabbits showed a diminished or abolished righting reflex (Cogan 1940, 1943). The present strain of waltzers also exhibited an impaired righting reflex. Ageing did not seem to influence the test performance of the waltzers.

Normal galvanic responses in combination with abolished reaction to rotation have been reported in hereditary deaf mice (e.g. Quix 1907, Gruneberg 1943). Such a combination was also found in the waltzers irrespective of age.

Scarpa's ganglion had a normal appearance when examined under the light microscope. Electron microscopic studies may, however, reveal degenerative changes, most likely in the older animals.

Vestibular lesions similar to those described above have been produced in cats by means of streptomycin intoxication (Wersall & Hawkins 1962). Pronounced degeneration of the vestibular neuroepithelium was found with the disappearance of all type I hair cells and most of type II. The cats developed a circling habit, the righting reflex was gradually abolished and per- and post-rotatory nystagmus was no longer elicitable. The galvanic response, however, remained unchanged.

These observations indicate that galvanic stimulation cannot be used as a test for peripheral damage, whether endogenous or exogenous, of the vestibular neuroepithelium.

CONCLUSION

Conventional testing produced no vestibular response in the waltzing guinea pigs irrespective of their age. The galvanic test may not be included here as it does not reflect the condition of the peripheral vestibular apparatus. However, the fact that an extremely powerful peripheral stimulus, such as a combination of fenestration and calorization, elicited a nystagmus response in the young waltzers shows the presence of functioning vestibular end organs. Animals aged more than 1 1/2 years did not respond to this mode of calorization.

Thus, the waltzing guinea pigs of this strain are born with a severely decreased peripheral vestibular function. This impairment was evidently progressive in character, presumably until total loss of function occurred. Further more, since it was possible to induce peripherally a nystagmus response in the young animals, there could be no discontinuity in the vestibular reflex arc.

The results are in agreement with the morphologic evidence (Ernstson et al. 1969, 1970): the vestibular neuroepithelium underwent progressive degeneration, discernible already at birth. However, most of the hair cells, both type I and type II (Wersall 1956), apparently existed at birth. The afferent and efferent nerve endings showed no changes until the hair cells had disintegrated.

ZUSAMMENFASSUNG

Die Physiologie des Vestibularisapparates wurde bei einem Stamm Meerschweinchen mit dominant erblichem walzenden Gang untersucht. Bei Rotation der Tiere wurde kein Nystagmus ausgelöst und Neigung zur Seite rief keine Augenbewegung in entgegengesetzter Richtung hervor. Bei jungen walzenden Meerschweinchen konnte man nach Fenestrierung eines Bogenganges und Spülung der Bulla mit heissem oder kaltem Wasser einen kalorischen Nystagmus auslösen. Bei älteren Tieren blieb solche Reaktion aus. Bei walzenden Tieren jeden Alters war die Fähigkeit zu *schwimmen herabgesetzt und der Umdrehungsreflex* erschwert, was bei Verhaltensstudien gezeigt wurde. Galvanische Reizung rief bei Tieren jeden Alters eine normale Reaktion hervor. Das Ganglion Scarpae sah bei lichtmikroskopischer Untersuchung normal aus. Man zieht den Schluss, dass die Vestibularfunktion dieses Stammes walzender Meerschweinchen schon bei der Geburt herabgesetzt ist, was mit zunehmendem Alter bis zu vollständigem Funktionsverlust fortschreitet. Dieses Ergebnis steht im Einklang mit früheren Berichten über eine fortschreitende neuroepitheliale Entartung im Vestibularisapparat dieser Tiere.

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HISTOCHEMISTRY OF THE NORMAL EUSTACHIAN TUBE

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Abstract Histochemical studies of various cellular components of the normal eustachian tube have been undertaken in the guinea pig cat and squirrel monkey Oxidative and hydrolytic enzymes were strongly active in ciliated columnar cells less active in goblet cells suggesting active metabolism In general cellular components of the subepithelial layer reacted strongly to the various enzymes Of the glands studied serous cells contained more oxidative enzymes than did mucous cells Within the hyaline cartilage the chondrocytes demonstrated fairly strong enzyme reactions the intercellular matrix none These findings assist our understanding of normal eustachian tube physiology regarding ciliary clearance and lubrication and serve as a basis for understanding inflammatory diseases such as the middle ear effusions

Eustachian tubes of mammals have not been extensively studied with modern methods of to and cytochemistry The function of the stachian tube is 1) to channel air from the nasopharynx to the middle ear thereby maintaining equal air pressure in the middle ear and environment and 2) as a drainage route for foreign materials through the action of ciliated epithelial cells which line the lumen of the eustachian tube Embryologically the epithelial lining of the eustachian tube is an extension and modification of upper respiratory tract mucosa

It is known that the lumen of the eustachian tube is lined with ciliated columnar cells and goblet cells which are usually resting upon basal cells The subepithelial layer rests on hyaline cartilage or bone and contains connective tissue cells and fibers lymph and blood vessels nerve fibers and glandular elements

The purpose of this study is to observe histochemically the activity of various enzymes in the eustachian tube of the guinea pig cat and squirrel monkey and to discuss implications in terms of eustachian tubal function

MATERIAL AND METHODS

Animals used in this study were 6 healthy adult guinea pigs 3 cats and 6 squirrel monkeys After decapitation under anesthesia by pentonembutal injection the temporal bones were immediately removed with the eustachian tube attached Then the tissue blocks with the exception of oxidative enzymes were fixed in 4° neutral formol-calcium or 10 % neutral formalin solutions for 24 hours at 4 C Fixed specimens were decalcified for 4 to 7 days at 4°C in a 10% buffered solution of EDTA at pH 7 45 (Balogh 1962)

Decalcified tissue blocks were frozen mounted and cut serially at 15–20 μ in a cryostat (−30°C)

The sections were mounted on clear cover glasses thawed slowly dried at room temperature for 20–30 minutes then incubated in one of the substrate solutions designed for demonstration of the activity of the following enzymes malic dehydrogenase and succinic dehydrogenase diphosphopyridine nucleotide

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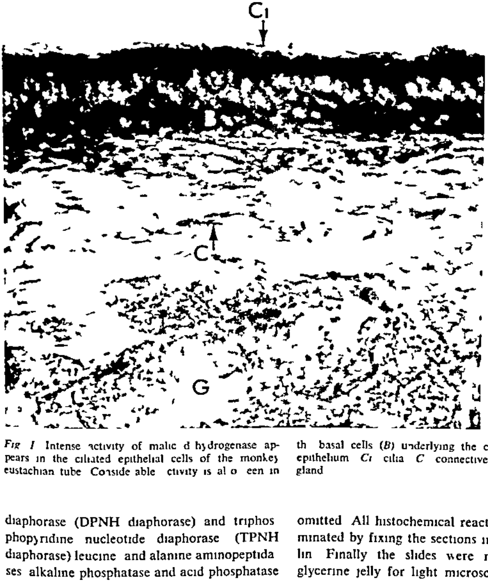


Fig 1 Intense activity of malic dehydrogenase appears in the ciliated epithelial cells of the monkey eustachian tube Considerable activity is also seen in the basal cells (B) underlying the ciliated columnar epithelium Ci cilia C connective tissue cell G gland

diaphorase (DPNH diaphorase) and triphosphopyridine nucleotide diaphorase (TPNH diaphorase) leucine and alanine aminopeptidases alkaline phosphatase and acid phosphatase and non specific esterase

Techniques used for the demonstration of the various aforementioned enzymes were identical to those employed in a previous study (Paparella et al 1970)

Controls for the histochemical reactions were made by incubating sections in media from which the respective substrate had been omitted All histochemical reactions were terminated by fixing the sections in 10 formalin Finally the slides were mounted with glycerine jelly for light microscopy examination

RESULTS

Epithelial layer

The epithelium of the eustachian tube is mainly composed of three types of cells pseudostratified columnar cells which usual bear cilia on the free surface of the c plasm goblet cells which are

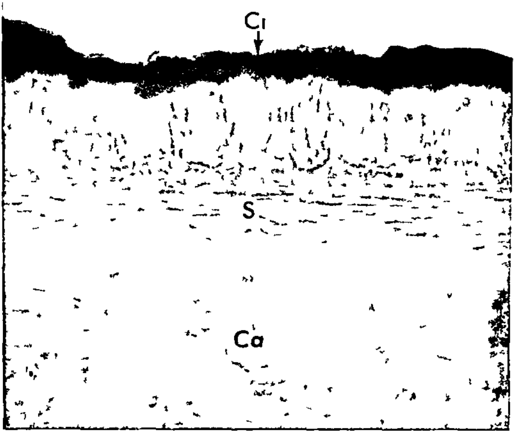


Fig 2 Strong alkaline pho phatase activity is found ly in the cilia (*Ci*) of ciliated epithelial cells of cat subepithelial connectiv tissue *Ca* cartilage

among the ciliated columnar cells and basal cells which underly the aforementioned two superficial cells

Ciliated columnar cells of all species showed a strong activity for succinic dehydrogenase malic dehydrogenase (Fig 1) lactic dehydro genase DPNH diaphorase and TPNH dia phorase especially in upper areas of cyto plasm

On the other hand goblet cells were much less reactive to oxidative enzymes than were ciliated columnar cells Deformazan granules were deposited in their cytoplasms especially below the nuclei In the guinea pig and the cat the goblet cells were much more fre quently seen in the epithelial lining of the eustachian tube than in that of the squirrel monkey The basal cells contained moderate amounts of deformazan deposits throughout the cytoplasm

Strong acid phosphatase activity was noted in the ciliated cells of the squirrel monkey especially in the apical area of cytoplasm However cilia bearing columnar cells of cat and guinea pig revealed moderate amounts of azo dye granules in the apical cytoplasm Goblet cells reacted faintly in all species Simi larly basal cells displayed feeble activity



Fig 3 The ciliated columnar cells of the monkey eustachian tube show a strong activity of non specific esterase especially in the supranuclear cytoplasms This enzyme activity is also seen as a dot (arrow) around the basal cells (*B*) *Ci* cilia *S* subepithelial connective tissue

No alkaline phosphatase was found throughout the entire epithelium of the eustachian tube except for cilia in the cat Cilia of the cat showed remarkable activity of this enzyme (Fig 2) while those of the guinea pig and the squirrel monkey did not reveal any enzyme activity Occasionally positive cells were observed but they were probably migrating leukocytes

A strong activity of non specific esterase was present in the ciliated columnar cells of the cat and squirrel monkey (Fig 3) Intracytoplasmic localization of azo dye deposits seemed to be concentrated in the supranuclear portion while goblet cells and basal cells reacted weakly

The distribution pattern of leucine alanine aminopeptidases were almo

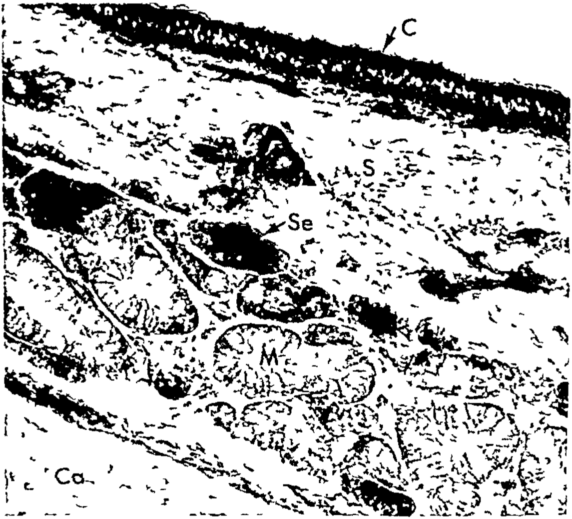


Fig 4 Hi h enzyme reaction for DPNH diaphoras rs in the serous cells (*Se*) of mixed glands of irrel monkey while only w ak enzyme response appears in th mucous cells (*M*) Ciliated columnar epithelium also shows intense enzyme activity *C* cilia *S* subepithelial connective tissue *Ca* cartilage

cal Marked enzyme activity was noted in the ciliated columnar cells of the squirrel mon key but less enzyme reaction was detectable in the guinea pig and the cat Only a slight activity was seen in the goblet cells of all species Basal cells contained considerable amounts of chelating azo granules

Cuboidal and squamous cells usually found in the transitional areas of the tympanic cavity showed moderate to weak activity for the various enzymes

Subepithelial layer

Connective tissue cells such as fibrocytes or fibroblasts and the endothelial cells of the small blood vessels reacted strongly to various oxidative and hydrolytic enzymes Fibro ytes and fibroblasts of all species showed a con siderable amount of diformazan deposits throughout the cytoplasm These cells ap peared to be mostly located near the base ment membrane of epithelial lining and junc tional areas between connective tissue layer and cartilaginous tissue Collagen fibers re mained negative for the oxidative enzymes Strong alkaline phosphatase activity was seen in the walls of small blood vessels of all ani mals and sometimes in the perichondrium of the guinea pig and cat

Some types of connective tissue cells con



Fig 5 Serous c lls (Se) of monkey mixed glands display strong TPNH diaphorase activity compared with mucous cells (M)

tained large amounts of acid phosphatase and non specific esterase Presumably they were immature fibroblasts or wandering leukocytes

A marked reaction to aminopeptidases was demonstrated exclusively in the cellular com ponents of the connective tissue layer This probably occurred in fibroblasts and some fi brocytes

Glandular elements

There were considerable numbers of glandular elements in the subepithelial tissue of the guinea pig cats and squirrel monkeys These were mixed serous and mucous glands which were usually distributed near the pharyngeal orifice They became sparse as the mucosa



Fig 6 Alkaline phosphatase activity was confined to the annular tissues (arrow) surrounding acini (*A*) of monkey mixed glands

became more and more attenuated from the orifice upward The histochemical response for various oxidative and hydrolytic enzymes was almost identical for all animals

Generally serous cells of mixed glands contained more oxidative enzymes than did mucous cells Considerable amounts of diformazan granules while slightly noted in the mucous cells were diffusely deposited in the serous cells in the area below the nuclei DPNH diaphorase and TPNH diaphorase were strongly identified in the serous cells but appeared weak in the mucous cells (Figs 4 5) Acid phosphatase and non specific esterase activities were almost identical in reaction to the above However alkaline phosphatase activity was confined to the annular tissues surrounding acini and to the capillaries (Fig 6) Glandular cells were moderately to weakly positive for aminopeptidases



Fig 7 Marked activity of TPNH diaphorase is seen in the chondrocytes (*Ch*) of hyaline cartilage and ciliated columnar cell in the guinea pig eustachian tube No histochemical reaction is present in the intercellular cartilaginous matrix (*I*) *B* basal cell *S* subepithelial connective tissue

Cartilage

This portion of the tube consists of chondrocytes and intercellular cartilaginous matrix The histochemical distribution pattern in cartilage in the guinea pig cat and squirrel monkey was almost the same Moderate to strong activity of succinic dehydrogenase malic dehydrogenase lactic dehydrogenase DPNH diaphorase and TPNH diaphorase was seen the chondrocytes but no activity wa

trable in the intercellular cartilaginous matrix (Fig 7)

On the other hand little activity of acid phosphatase non specific esterase and amino peptidases was noted in the chondrocytes In tercellular matrix remained negative for these hydrolytic enzymes

DISCUSSION

Although some anatomical interspecies dif ferences exist in the eustachian tube of the guinea pig cat and squirrel monkey cellular components of the tubal lumen are funda mentally similar

The mucous membranes forming the real lumen of the tube consists mainly of a ciliated columnar epithelial layer and a thin sub epithelial layer Our present histochemical findings prove that cilia bearing cells in the eustachian tube possess active oxidation reduc tion metabolism at the levels of Krebs tri carboxylic acid cycle and of the cellular respiratory chain In addition the consistent appearance of hydrolytic enzymes in the cili ated cells of the tube also indicates active metabolism

Similarly in the middle ear of some mam mals we have also noted high levels of vari ous oxidative and hydrolytic enzymes mainly in the ciliated epithelial cells (Hiraide & Pa parella 1971) Cress et al (1965) have also demonstrated a high activity of oxidative en zymes in the ciliated respiratory epithelium Recently several investigators have reported the undoubted existence of evacuating mech anisms in both the middle ear and the eustachian tube through experiments (Holm gren 1934 Compere 1958 Rogers et al 1962 Sato 1939) Following the application of vari ous foreign materials such as lipoidal and fluorescent materials and starch into the middle ear cavity they observed rapid clear ance of these materials from the middle ear to the nasopharynx through the eustachian tube According to Rogers et al (1962) fluores cent material introduced into the middle ear cavity appeared in the pharynx at varying intervals from immediately to minutes after instillation In no instance did the dye appear after 10 minutes had elapsed

It is of interest to note that intense alkaline phosphatase activity was observed in the cilia of ciliated epithelial cells of the cat whereas the cilia of guinea pig and squirrel monkey did not display any enzyme activity The same finding was also noted in the ciliated cells of the middle ear of the cat (Hiraide & Paparella 1971) This indicates that the distribution pattern of this enzyme in animal tissue is variable from species to species, as has been reported in various organs by several investigators (Kagawa & Osanai 1963 Sarkar & Deb 1965) It is generally known that cilia function only when moistened Under adequately moist conditions the cilia beat powerfully and rapidly in the direction of flow and the mucous blanket containing foreign material and other substances is carried toward the pharynx by cilia

There were varying numbers of goblet cells among the ciliated surface cells which supply a portion of secretion According to Lim et al s observation (1967) in the tubal epithelial lining of guinea pig there are no other secretory cells such as dark granulated cells and intermediary cells except goblet cells

On the other hand as Rudinger (1870) described in many mammals the subepithelial layer of the eustachian tube contains glandular elements which open through the epithelial layer The staining characteristics of the glands as revealed by histological and histochemical techniques indicate that they are composed of two types of cells namely serous cells and mucous cells as are most glands of this type throughout the respiratory tract

In the present study glandular cells also contained considerable amounts of various enzymes These findings resemble to some degree the enzyme distribution pattern in and functioning salivary glands These glands obviously supply lubrication for the eustachian

tube and posterior pharynx as do the goblet cells

These histochemical studies have shed additional light on cell function of the normal eustachian tube of three animal types and serve as a background for further studies Ciliated cells as they relate to a drainage function and secretory and gland cells as they relate to a lubrication function have been alluded to What role these cells play and what their histochemical correlates are in diseased inflammatory conditions such as middle ear effusions remains to be determined.

ACKNOWLEDGMENTS

We ar grateful to Mr Hans Erikkson for his technical assistance and to Mrs Ann Crowell for photography

ZUSAMMENFASSUNG

Histoch mische Untersuchungen verschi dener Zell gruppen in der eustachischen Tube gesunder Meer schweinchen Katzen and Totenkopfaffchen wurden unternommen. Oxyd erende und hyd olysierende Fermente zeigt n eine starke *Aktivitat im Flimmer* epithel aber eine geringere Aktivitat in den Be her zellen. Die Zellen im Bindegeweb zeigten, dass diese Fermente haufig vorkommen Die Versuche zeigten, dass d e oxydierenden Ferm nte vor allem in den serosen Drusen zu finden waren Nur die Chondrozyten hatten im Hyalinenknorpel eine nennenswert Fermentreaktion Diese Beobachtungen mogen dem Verstandnis der normal n Physiologie der eustachi schen Tube dienen und zwar in Bezug auf den ci liaren Transport und des en Lubrikation in Hinsicht auf die Entwicklung der Entzundungen wie Flussig keitsausschwitzungen im Mittelohr

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THE NORMAL FUNCTION OF THE EUSTACHIAN TUBE

A Study of 102 Cases

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Abstract A method permitting quantitative tubal function tests across an intact tympanic membrane has been worked out The method permits a continuous recording of the volume deviation of the drum in relation to the neutral position both at changes of the ambient pressure and changes of the pressure in the middle ear Repeated testings have been performed on 102 cases with normal ears which all had normal hearing i e a normal audiogram and normal ear findings 64 % could equilibrate under dynamic pressure conditions i e simulated descent 84 could propagate a square wave pressure from the rhinopharynx to the ear and 79 had a positive Toynbee s test 72 could equilibrate negative ear pressures completely while the rest have a reduced capacity to equilibrate or none at all though the ears were regarded as normal The findings are discussed

The aim of this investigation was to find out what is meant by a normal tubal function and , apply several different tests to the same person Further a comparison is made between these tests and we have tried to decide which one is best suited for routine clinical work The function of the Eustachian tube has been studied from different aspects in 102 individuals with normal ears When selecting the subjects it is assumed that the Eustachian tube functions normally if the subjects have normal hearing as tested by audiogram normal otoscopy including otomicroscopy and normal mobility of the drum and have no history of ear disease nor subjective or objective signs of catarrhal infections

This investigation was supported by grants from the Swedish Medical Research Council (No B70-14X 99-06 and B71-40X 99-07)

In the literature there is no material concerning people with thoroughly examined normal ears that have been tested in this way We think it is very important that the ear should be intact and the normal physiology undisturbed We have used a method by which the drum is in free communication with the surrounding atmosphere during the test

This work is a part report in a series of papers about middle ear mechanics (Ingelstedt et al 1967 Elner et al 1971)

METHODS FOR TESTING THE EUSTACHIAN TUBE FUNCTION ACROSS AN INTACT TYMPANIC MEMBRANE

Earlier Methods

A *Methods for recording the displacement of the drum*

1 Microscope inspection of the drum movements when air is passing through the tube (Krassnig 1935 Zollner 1942 Adams 1953)
2 Determination of air passage through the Eustachian tube by auscultation in the ear canal (Toynbee 1853 Lucae 1867 von Gyergyay 1932 Zollner 1942 Perlman 1943)
3 Closed ear canal manometry (Politzer 1861 1862 Woyatschek 1908 von Gyergyay 1932 Terkildsen 1956 Oltersdorf 1954

1962 Kottmeyer 1958 Juganov 1960 Flisberg et al 1963 Riu et al 1966 Siedentop et al 1968)

4 Quantitative determination of the volume displacement of the drum by flow volume meter (Ingelstedt & Jonson 1967 Ingelstedt et al 1967)

B *Methods for recording the middle ear pressure change after air passage through the Eustachian tube*

1 The impedance method (Metz 1946 Thomsen 1955 1957 Terkildsen & Scott Nielsen 1960 Holmquist 1969 Liden et al 1970)
2 The pneumophone method (v Dishoeck 1937 1939 1941 1947 1952 Perlman 1943 among others)

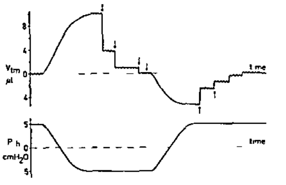


Fig 1 Equilibration of static relative over and underpressure Upper curve illustrates the recorded volume displacement of the drum outwards (+) and inwards (−) Arrows mark deglutitions Lower curve pressure change in the pressure chamber and ear canal

The Present Method

Our aim has primarily been to study the pressure regulating capacity of the Eustachian tube during and after changes of the ambient pressure Physiological tests imply a free air communication between the outside of the drum and the ambient air A method permitting studies of this kind has been worked out (Elner et al 1971)

The volume displacement of the drum V_{tm} in relation to its neutral position can be recorded both at a change of the ambient pressure P_h and of the middle ear pressure P_m The neutral position of the drum is determined directly when the tubal function is good and indirectly when it is poor When the actual volume position of the drum is determined in relation to its neutral position the pressure across the drum P_{tm} is calculated with the aid of the volume pressure diagram of the tympanic membrane system $V_{tm} = f(P_{tm})$ (Elner et al 1971)

PERFORMANCE OF THE TESTS

1 *Equilibration of static pressure differences across the tympanic membrane*

By changing the ambient pressure P_h with 10 cm H_2O a pressure difference is set up across the drum P_{tm} and a static over and under pressure in relation to the ambient pressure is established in the middle ear cavity The drum thereby moves outwards or inwards (Fig 1) The subject is then allowed to equilibrate the pressure difference by deglutitions From the recorded V_{tm}-curve the pressure equilibration across the drum P_{tm} can be calculated as an expression of the pressure equilibrating capacity of the Eustachian tube (Elner et al 1971) We have chosen to test the tubal passage at chamber pressure changes of 10 cm H_2O in order not to exceed the limit for the relative overpressure which passively forces the tube open During each equilibration the number of deglutitions has been limited to 10 and the time interval between each deglutition has been approximately 20 sec This interval has been chosen in order to avoid any strain on the pharynx muscles The equilibration test has been repeated twice Equilibrations have been made by deglutition but in three cases the subjects have equilibrated more effectively by movements of the mandible and have been allowed to use this method The subjects have been swallowing dry but in all cases with reduced function of the Eustachian tube water swallowing has also been tried

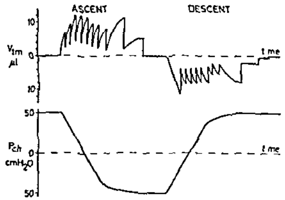


Fig 2 Equilibration of dynamic relative over and under pressure ascent and descent Upper curve illustrates the recorded volume deviation of the drum outwards (+) and inwards (−) Lower curve pressure change in the pressure chamber and ear canal

2 *Equilibration of dynamic pressure differences across the tympanic membrane*

The volume displacement of the drum V_{tm} is recorded during changing chamber pressure, 'ascent and descent, from +50 to −50 cm H_2O or the reverse in 25 sec during which time the subjects are told to swallow as many times as possible (Fig 2) From the recording of the drum volume displacement the mean pressure across the drum is calculated for the whole pressure change period as an expression of the equilibration capacity of the tube (Elner et al 1971) These tests are repeated at least twice on every subject with a time interval of 3 min

During these tests we have also determined the level of the relative overpressure in the middle ear at which the tube is passively forced open

3 *Toynbee's test*

The pressure in the rhinopharynx and the possibly resulting volume displacement of the drum are recorded simultaneously during deglutitions with the nose clamped The pressure in the middle ear in relation to the ambient pressure is always the same when the manoeuvre begins The test is repeated twice and the number of deglutitions has been limited to 5 for each test

Table I *The distribution of the material*

Age	Men	Women
21–30	58	33
31–40	2	2
41–50	3	1
51–60	3	—
Total	66	36

4 *Valsalva's test*

The pressure in the rhinopharynx and the resulting volume deviation of the drum are recorded simultaneously when the pressure in the upper airways is increased and the nose is clamped The subject is trained to produce a pressure in the rhinopharynx always exceeding +30 cm H_2O The test is repeated five times

5 *Propagation of over and underpressure of square wave type from the rhinopharynx to the middle ear by deglutition*

Pressures in the rhinopharynx of ±5 and ±10 cm H_2O have been used Three deglutitions have been made at each pressure level where after the subject equilibrates the resulting middle ear pressure before a new pressure is applied to the rhinopharynx If the subject cannot equilibrate completely the ambient pressure is adjusted to the prevailing middle ear pressure before a new test is begun (Elner et al 1971)

All the tests described above are performed with the subject in the sitting position

MATERIAL

The material consists of 102 subjects 66 men and 36 women all volunteers recruited from blood donors and students The age distribution appears from Table I The group between 21 and 30 years predominates which is explained by the large group of students involved In all cases there was a negative history of airway diseases and normal ear findings The hearing limit of the audiograms was always within 0–20 dB (related to ISO standard 1964)

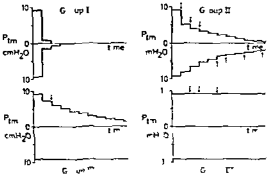


Fig 3 Grouping of the material with regard to equilibration capacity of static relative overpressure $+P_{tm}$ and underpressure $-P_{tm}$ Arrows indicate deglutitions

All the subjects were without signs of catarrhal infection subjectively as well as objectively at nose and throat examination

RESULTS

Equilibration of static pressure differences across the tympanic membrane

The material has been divided into four groups according to the ability to equilibrate a static relative over and underpressure in the middle ear (Fig 3) Group I equilibrates pressure differences across the drum completely group II equilibrates with small residual pressure in the middle ear group III is only capable of equilibrating relative overpressure but not underpressure and group IV is incapable of equilibrating any pressure over as well as underpressure In this material there are four cases of tuba aperta without symptoms and these are assigned to group I

Distribution of the material appears from Table II and this division will be the basis

Table II *The distribution in different tubal function groups*

Distribution	No of cases	
Group I	74	72
Group II	21	21
Group III	2	2
Group IV	5	5
Total	10[illegible]	100

of the comparison and assessment of the other results Furthermore the cases in group I have been analysed with regard to the number of deglutitions necessary for complete equilibration (Table III)

Comment Two attempts at equilibration have always been made at the relative over and underpressure in the ear There is a good correlation between the results of the two tests In all cases within groups III and IV swallowing of water was also tested without improvement All cases in groups III and IV were tested a second time several months after the first examination These later tests gave the same results as the previous ones

Table III *Distribution of cases in tubal function group I according to the number of deglutitions necessary for complete equilibration*

	No of cases	
Group I*a* tuba aperta	4	4
Group I*b* 1–3 equilibrations	33	32
Group I*c* 4–10 equilibrations	37	36

Equilibration of dynamic pressure differences across the tympanic membrane

Comparative tests between ascent and descent have been made at least twice on 39 subjects who all belonged to group I*b* and I*c* (Fig 2) The mean pressure across the tympanic membrane Ptm_{mean} has also been calculated for a complete ascent and descent respectively and both these pressures have been compared (Fig 4) Every point in the diagram represents Ptm_{mean} for a single subject The diagram is so constructed that if the subject s capacity to equilibrate dynamic over and underpressure was the same the Ptm_{mean} relation would fall on the dotted line It is clear from the figure however that nearly all cases have a better capacity to equilibrate dynamic overpressure than underpressure There is no similar difference when these individuals in group I are tested with static over and underpressure

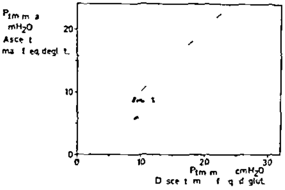


Fig 4 Relation between the mean pressure across the tympanic membrane at ascent and descent with a maximum of deglutitions

Comment From the comparison above it is clear that descent puts the person s capacity for active equilibration to a more severe test than ascent This difference is not found under static pressure conditions

In the following study we have therefore chosen to concentrate on the capacity for equilibration during descent This test has been made on 89 subjects Out of these 56 (63) were capable of equilibration while the rest lacked this ability On each subject able to equilibrate the mean pressure across the drum Ptm_{mean} has been calculated for the descent The results in Table IV support earlier division into tubal function groups static pressure conditions

For those who can equilibrate completely during these extreme conditions of ambient pressure changes Ptm_{mea} seems to be a quantitative measure of the equilibrating capacity of the tube

At ascent the tube is forced open passively on all the subjects tested 79 in all when the relative overpressure in the middle ear P_{tx} reached a certain pressure level called forcing pressure At continued decrease of the chamber pressure after the passive opening moment of the tube we get different types of pressure regulation across the tube In 69 cases the mean value of the forcing pressure was +41 9 cm H_2O (S D ±15 8 S E ±1 9) whereafter the position of the drum during the continued decrease of the chamber pressure was recorded in a constant position outside the neutral position i e P_{tm} is constant (Fig 5*a*) This means that gas is continuously leaking out from the middle ear to the rhinopharynx through the tube The mean value of this constant P_{tm} in the 69 cases during the leaking phase was +18 3 cm H_2O (S D ±11 3 S E ±1 4) On the other 10 cases a serrated V_{tm} curve was recorded (Fig 5*b*) which means that the tube opens and closes several times during the continued decrease of the chamber pressure

Comment We cannot find any relation between the tubal function according to groups I–IV and the magnitude of the recorded forcing pressure as seen from Table V

Toynbee s test

This test was positive in 74 out of 94 cases (79) The pressure wave in the rhinopharynx during deglutition is in most cases biphasic with a primary positive and a secondary negative phase The tube opens and closes

Table IV *The equilibrating capacity and the mean pressure across the tympanic membrane during descent in relation to the different tubal function groups*

Maximum frequency of deglutitions Descent −50→+50 cm H_2O/25 sec

Tubal function groups	Pos /No		Ptm_{mean} cm H_2O Mean	S D	S E.	No
Group I*b*	28/28	100	−10	±2 4	±0 5	28
Group I*c*	26/34	77	−14	±5 4	±1 1	26
Group II	2/20	10	−32	±4 2	±3	2
Groups III–IV	0/7	0	—	—	—	—
Total	56/89	63	−12 4	±6 0	±0 8	56

Table V *The pressure necessary to force the tube open during ascent in relation to the different tubal function groups*

Tubal function groups	Forcing pressure P_{tm} cm H_2O Mean	SD	SE	No
Group I	+46 6	±17 7	±2 4	56
Group II	+37 2	±11 5	±2 8	17
Group III	+38 0	± 9 9	±7 0	2
Group IV	+36 4	±10 7	±5 3	4
Total	+43 9	±16.5	±1.9	79

during the negative phase in 47 cases the resulting pressure in the middle ear being negative In 18 cases there was an alternately positive and negative middle ear pressure while in 9 cases the test constantly gave a positive middle ear pressure (Table VI)

Comment It is clear from Table VII that reduced tubal capacity according to groups III–IV gives a neg tive Toynbee s test in all cases The two cases in the tubal function group I incapable of performing a positive Toynbee s manoeuvre could only equilibrate by moving the mandible but not by deglutition

Valsalva s test

This test has been performed in 101 cases Out of these 86 (86) were able to force air into the middle ear The pressure in the rhinopharynx during the manoeuvre always exceeded +30 cm H O

Comment We find no relationship between the tubal function according to groups I–IV and the Valsalva test (Table VII)

Propagation of over and underpressure of square wave type from the rhinopharynx to the middle ear by deglutition

There was tubal passage in 61 out of 73 cases (84) There is no difference in tubal passage if the pressure is positive or negative or 5 or 10 cm H O

Comment There is agreement between earlier results in the tubal function groups and the capacity to propagate the rhinopharynx

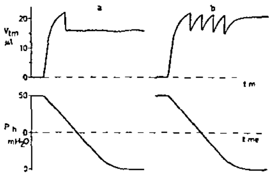


Fig 5 Two different examples of V_{tm} recording Upper curve (*a*) when the tube is continuously leaking the volume position of the drum is constant Upper curve (*b*) the tube opens and closes alternately and the curve is serrated Lower curves pressure change in the pressure chamber

pressure to the middle ear (Table VII) The two cases in tubal function group I who lacked ability to propagate the rhinopharynx pressure by deglutition could only equilibrate during static pressure conditions by moving the mandible The reason why only 73 cases out of 102 have been examined is that the test was added only after this study was started

DISCUSSION

The method of equilibrating a static relative underpressure in the ear has been described by Flisberg et al (1963) and is called the aspiration method The starting point i e a relative underpressure in the middle ear can be achieved in two ways The ambient pressure in the pressure chamber can be raised or a negative pressure of a square wave type can be applied to the rhinopharynx and propagated to the middle ear during deglutition The resulting

Table VI *Middle ear pressure after Toynbee s test*

Resulting middle ear pressure	No of cases	
Negative	47	64
Alternately pos and neg.	18	24
Positive	9	12
Total	74	100

Table VII *Results of different tests in relation to the tubal function groups*

Tubal function groups	Square wave pressure Pos /No		Toynbee Pos /No		Valsalva Pos /No	
Group I	50/52	96	67/69	97	63/73	86
Group II	11/16	69	7/18	39	16/21	76
Group III	0/2	0	0/2	0	2/2	100
Group IV	0/3	0	0/5	0	5/5	100
Total	61/73	84	74/94	79	86/101	86

relative underpressure is thereafter equilibrated by deglutition If these two techniques are compared 93 (groups I II) will be able to equilibrate the relative underpressure in the middle ear after increase of the chamber pressure Corresponding relative underpressure is only propagated from the rhinopharynx to the middle ear in 84 but when this underpressure is established in the middle ear all are able to equilibrate (Table VII)

The only material on which the aspiration method has been used across an intact tympanic membrane has been published by Holmquist (1969) With the aid of a negative square wave pressure in the rhinopharynx (−10 to −25 cm H_2O) during deglutition he created an underpressure in the middle ear in 20 normal Thereafter he studied the ability to librate with the impedance method He found that all cases could normalize the middle ear pressure already after 1–2 deglutitions These 20 cases correspond to our cases in group I*b*

As in the case of aspiration a static relative *overpressure is achieved in two different ways* either through a decrease of the ambient pressure or through a positive square wave pressure in the rhinopharynx which is propagated during deglutition to the middle ear and thereafter equilibrated 95 (group I II III) could equilibrate a relative over pressure in the middle ear created by decrease in the chamber pressure and 84 could propagate a positive rhinopharynx pressure into the middle ear All these later cases could however equilibrate the resulting overpressure

Thomsen (1957) studied the ability of 100 normal persons to equilibrate a relative over pressure in the middle ear recorded by the impedance method He found that 73 % could equilibrate completely 19 could reduce the pressure to less than 5 % of the original value while 8 could not equilibrate at all If the first two groups are rolled into one they correspond to our groups I–III and make 92 The results are thus well in accordance with ours (95 %) in spite of the fact that Thomsen s examinations were performed in a recumbent position suitable for technical reasons In view of this circumstance Thomsen s results are rather remarkable since we know that the tubal function is impaired in the recumbent position (Perlman 1939 Ingelstedt et al 1967 Rundcrantz 1969)

When testing with positive and negative pressures in the rhinopharynx at deglutition we have used ±5 and ±10 cm H O We have chosen +10 cm H O as the upper limit since we want to be sure not to reach a value that will force the tube open passively We found that 84 % of the cases were positive Zollner s *material (1942) covered 200 ears Out of these* 75 opened the tube at pressures between 0–10 cm H_2O while the rest needed higher pressures The examination was made in the sitting position Thomsen (1957) found that out of 100 cases 83 opened the tube between 0–10 cm H_2O and 16 % between 15–30 cm H O Riu et al (1966) examined 308 ears 72 opened the tube at a pressure in the rhinopharynx of 0–15 cm H_2O 23 between 15–30 cm H O and 5 % at pressure higher than 30 cm H_2O If this French material is

considered it will be found that the number of examined cases that open at a positive pressure in the rhinopharynx of 0–10 cm H_2O is much smaller than the number opening at the pressure of 0–15 cm H O This figure is remarkably low as compared with ours and supports the opinion that the French material may not be quite normal It is also doubtful if Zöllner s material can be considered quite normal since it consists of pilots and pilot aspirants

The Toynbee test was positive in 79 By way of comparison Zöllner points out that 70 of people with a normally functioning Eustachian tube perform a positive Toynbee s test In a corresponding investigation Thomsen found that 72 of the cases were positive We agree with Zollner that the tubal function is normal when Toynbee s test is positive The better the tubal function the more positive Toynbee s test (Table VII) The test is positive in 97 in group I and negative in all cases in groups III–IV

There is no comparison in earlier materials between different tests on one and the same individual There are thus several factors which distinguish earlier reported materials from the normal material which is now presented

What is to be regarded as a normal tubal function has been the subject of much discussion Opinions have varied depending on the investigator s technique material and so on After the aspiration method was described however this method has been more and more accepted as the most physiological one Provided that the individual is not subject to changes of the ambient pressure the function of the tube ought normally to be the equilibration of small relative underpressures in the middle ear caused by the absorption of oxygen and nitrogen which is continuously absorbed from the ear For normal hearing it is thus important to maintain a middle ear pressure near to or entirely corresponding to the ambient pressure In our material there are however seven cases (groups III IV) who during repeated tests with an interval of several months have a poor tubal function or none at all during deglutition but who nevertheless have normal hearing normal otoscopic findings and are subjectively and objectively without symptoms of catarrhal infection We have shown that some individuals unconsciously use another technique for equilibrating as for instance movement of the mandible yawning the Valsalva manoeuvre or blowing the nose It is thus important to know these alternative possibilities since they are otherwise easily classified as pathological

The aspiration method gives 93 positive cases (groups I–II) while 7 are not capable of equilibrating On comparison of the tests starting from a positive aspiration capacity as 100 a square wave pressure (±5 ±10 cm H O) from the rhinopharynx can be propagated to the middle ear by 90 and the Toynbee test is positive in 85 This indicates that the best way to decide whether the tubal function is normal or not is the aspiration method These former two tests have a good correlation to the tubal function according to Table VII

As regards the Valsalva test there is no correlation to the different tubal function groups (Table VII) This test is not a tubal function test in a physiological sense because there is no active muscle work in order to open the tube It is evident from the results however that all subjects who cannot equilibrate a negative middle ear pressure (groups III–IV) have a positive Valsalva s test This may be an important way for people lacking aspiration ability to remain without aural symptoms and has been verified on two cases in group IV

The testing of the equilibrating capacity during ambient pressure changes makes it possible to select people suitable for diving or flying Out of 93 persons with no experience of flying only 64 could equilibrate a dynamic relative underpressure by deglutition and in 3 cases by movements of the mandible The rest completely lacked this capacity and had to use the Valsalva manoeuvre to equilibrate

Equilibration of dynamic pressure changes as during descent, is thus the most difficult test It can only be performed by subjects in group I and even among these there is a highly significant difference ($P<0\ 001$) between groups I*b* and I*c* with reference to the mean value of the pressure across the drum (Ptm_{mean}) (Table IV)

ZUSAMMENFASSUNG

Eine Methode zur quantitativen Tubenfunktionsbestimmung uber ein intaktes Trommelfell ist entwickelt worden Sie ermöglicht eine fortlaufende Aufzeichnung der Volumenschwankungen des Trommelfells im Verhaltnis zur Neutrallage bei Druckveranderungen sowohl in der Umgebung als auch im Mittelohr Wiederholte Untersuchungen sind an *102* Versuchspersonen mit normalem Otoskopibefund und normalem Tonaudiogramm durchgefurt worden 64 v H konnten unter dynamischen Druckbedingungen d h bei Nachahmung von Tauchen einen Ausgleich erzielen 84 v H konnten einen Vierkantwellendruck vom Rhinopharynx zum Ohr weiterleiten und 79 v H hatten einen positiven Toynbeeschen Versuch 72 v H konnten negativen Druck im Ohr vollstandig ausgleichen wahrend die Druckausgleichsfahigkeit der übrigen trotz unauffalligem Ohrenbefund vermindert oder aufgehoben war

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ESTERASES OF POSTAURICULAR AND EAR CANAL SKIN COMPARED WITH CHOLESTEATOMA EPITHELIUM

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Abstract Esterase pattern of postauricular and ear canal skin of cholesteatoma epithelium and of granulation tissue was studied using electrophoretic and histochemical methods Esterase activity in the epithelium was localized in the granular cell layer The electrophoretic pattern of tissues was clearly different from that of serum In the epithelium the major activity was due to cholinesterase with a main fraction corresponding to the mobility of α globulin with additional fractions moving with α-globulins and with β_1 globulins Acetylcholinesterase was associated with α and β globulin fractions A weak α arylesterase and a stronger tissue esterase with a mobility of fast γ globulins were also demonstrated The electrophoretic analysis proved more informative than the histochemical method

In human skin the cholinesterases stain as a well-defined band in the granular cell layer adjoining the keratin layer (Wells 1960) In the connective tissue cholinesterases have been demonstrated in the cytoplasm of some of the stellate cells (Findlay 1955) Esterase action is also found in the sebaceous canal and round the hair shaft certain cells of the sweat gland acinus are positive and the duct lining cells show weak activity which increases towards the surface The exact part played by the esterases in the process of keratinization is not clear but evidently they are involved in the autolysis of the cellular content during which the superficial granular layer cells change into keratin mass (Jarrett & Spearman 1967)

Little is known at present of the histochemical differences between postauricular and ear canal skin but they clearly differ as regards clinical behaviour When transplanted at the bottom of the ear canal to form a new tympanic membrane the postauricular skin often is subject to continued exfoliation and dermatitis whereas ear canal skin adapts perfectly to its new position as ear drum The different genetic information (or enzymatic control) is also evident in the migration of keratin in the postauricular skin its direction is vertical to the skin surface but in the ear canal and on the tympanic membrane the migration is horizontal and the canals thus remain free of any accumulated keratin or extra wax (Litton 1968)

Some time ago we (Palva et al 1969 1970) studied the properties of postauricular and ear canal skin cholesteatoma membrane and granulation tissue as regards their lactate dehydrogenase (LDH) pattern It appeared that the isoenzyme pattern of these tissues differed markedly from the serum pattern the slow moving isoenzymes dominating in the former and the fast in the latter No significantly different pattern emerged when comparing the tissues examined On quantitative analysis actively growing granulation tissue showed the highest LDH values

In an earlier histochemical study (Palva et al 1970) we showed that in cholesteatoma epithelium there was a moderate cholinesterase activity in the granular cell layer which was

Table I *Frequency of esterase fractions in tissues*

Examined tissue	Esterase fraction 1	2	3 I	3 II	4	5	6
Cholesteatoma epithelium (N = 11)	–	–	7 (64)	11 (100)	6 (55)	2 (19)	10 (91)
Ear canal skin (N = 17)	–	–	15 (90)	17 (100)	14 (82)	1 (6)	13 (76)
Postauricular skin (N = 20)	–	–	19 (95)	20 (100)	15 (75)	6 (30)	19 (95)
Granulation tissue (N = 3)	–	–	3 (100*)	3 (100)	3 (100)	3 (100)	3 (100)

independent of the amount of covering kera tin In squamous epithelium without clinical cholesteatoma the activity was intense in the superficial 1/4 of the epithelium appearing as a net like black staining Columnar epithe lium showed no cholinesterase activity In the present study these enzyme analyses were extended to include not only cholesteatoma epithelium but also the postauricular and ear canal skin and the ordinary histochemical tissue staining technique was supplanted by *electrophoretic separation of various esterase* bands

MATERIAL AND METHODS

In connection with chronic ear surgery for holesteatoma a strip of postauricular and r canal skin was removed and a large piece f the cholesteatoma epithelium was obtained The specimens were first rinsed in sterile *saline dried with filter paper and kept frozen* at −20 C for a few days until the analyses could be made

In the initial experiments the tissues were first defrozen and then homogenized with a small amount of diluent but the electrophoretic analysis did not reveal any esterases not even in concentrated samples It was suspected that the esterases were not liberated from the cells and therefore after homogenization the *specimens were defrozen and frozen six times* If the specimen weighed more than 100 mg homogenization was carried out in an all glass homogenator adding 0.250 ml of distilled water For smaller specimens homogenization was done manually with a small glass homogenator by adding 20–40 μl distilled water In electrophoretic analysis 3 μl of the fluid was used

The electrophoretic separation of the ester ase bands was carried out using a method recently described (Palva et al 1970) How ever in addition to BW284c51 Mytelase was used for characterization of acetyl cholin esterases

RESULTS

Electrophoretic analysis

Table I shows the frequency of various esterase fractions in the tissues examined their electrophoretic mobility is seen in Fig 1 Frac tion 1 and 2 prealbumin and albumin arylesterase occurred in no specimen Frac tion 3 having the same electrophoretic mobility as α_1 globulin showed two distinct *bands and a subdivision into* bands 3 I and 3 II was made Fraction 3 II which was the slower appeared in 100 % of the examined tissues whereas fraction 3 I the faster one appeared almost constantly in the skin speci mens (90–100 %) and in all 3 granulation tissue specimens but somewhat less frequently in cholesteatoma epithelium (64 %) Fraction 4 showed somewhat different values in the epi thelial tissues but this was without statistical significance Fraction 5 was rare in all epithe lial tissues (6–30 %) but was present in the three granulation tissue specimens Fraction 6 on the other hand was a nearly constant find ing in all tissues

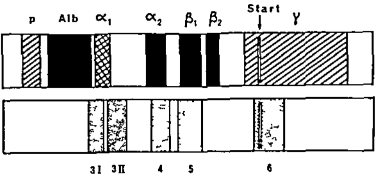


Fig 1 Schematic representation of the mobility of the 6 esterase fractions in the tissues as compared with normal protein pattern in serum

Fraction 3 II was found to be the dominant band in all tissues Next in order in the case of choles eatoma epithelium came band 6 in ear canal skin bands 3 I 4 and 6 (each equally often) in postauricular skin 3 I in 75° and band 6 in 25 ₒ (Table II)

No esterases whatever were demonstrated in four additional cases of extensive mastoid cholesteatoma in which the membrane was taken from the back wall of the cavity The membrane here was extremely thin and a substantial amount of keratin was inevitably in cluded

The characterization of the esterase bands was done using Eserine and Iso OMPA in concentration from 2 × 10 to 2 × 10 ³ and Mytelase in concentrations from 5 × 10 ⁰ to 5 × 10 ⁴ Fraction 4 was sensitive to both Eserine and Iso OMPA and thus consisted of cholinesterase (Fig 2) Band 3 II showed par tial sensitivity to Eserine and Iso OMPA as well as to Mytelase which suggests that it consisted partly of cholinesterase and partly of acetylcholinesterase Fraction 3 I was in most runs sensitive to Eserine but only partially sensitive in some in all runs it was partially sensitive to Iso-OMPA and Mytelase The main part of the fraction therefore seems to be cholinesterase and a minor part acetyl cholinesterase (Fig 3) These three inhibitors had a partial effect on fraction 5 which apparently also consists of cholinesterase and acetylcholinesterase Fraction 6 showed no clear inhibition by any of these inhibitors and the fraction can thus be regarded as one of the tissue esterases (Figs 2 3) Pherograms made of runs illustrated in Figs 2 and 3 with Vitatron densitometer are shown in Figs 4 and 5

Some inhibition tests were made with Myte lase combined either with Iso OMPA or Ese rine both combinations showed disappearance of fractions 4 and 3 I whereas the fractions 3 II and 6 remained in part in the epithelial specimens Thus fraction 3 II appears to con sist partly of α_1 arylesterase

Table II *Dominance of esterase bands*

1 = dominant 2 = next dominant

Examined tissue	Esterase bands 3I	3II	4	5	6
Cholesteatoma epithelium (N = 11)	2 (9)	1 (100)			2 (91)
Ear canal skin (N 17)	2 (33)	1 (100)	2 (33)		2 (33)
Postauricular skin (N = 20)	2 (75)	1 (100)			2 (25)

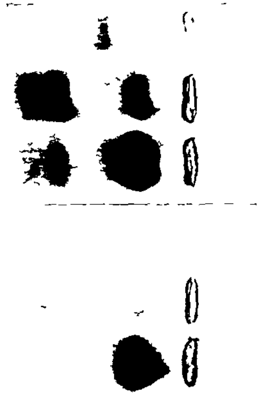


Fig 2 Electrophoretic analysis of NHS and of a homogenate of cholesteatoma epithelium and of post auricular skin (upper) subjected to inhibition test with eserine (10⁻⁴ M) for 30 min (lower) Marked inhibition of fractions 3 I 3 II and 5 and complete inhibition of fraction 4 is seen. The strong band for fraction 5 in cholesteatoma is due to hemoglobin

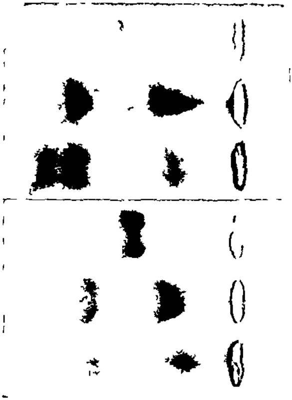


Fig 3 Electrophoretic analysis of NHS of a homogenate of nucleus caudatus and of postauricular skin (upper) subjected to inhibition test with Mytelase (10⁻⁴ M) for 30 min (lower) Partial inhibition of fractions 3 I 3 II and 5 is found

Normal human serum hemolysate of fresh erythrocytes, cadaver specimens of nucleus caudatus and commercial acetylcholinesterase were used as control specimens In the serum analysis fraction 4 cholinesterase and fractions 1 and 2 prealbumin and albumin arylesterase were demonstrated (Fig 2) while fractions 3 5 and 6 were absent Analysis of the nucleus caudatus homogenates disclosed two bands corresponding to 5 and 3 in the tissue analysis and both of them were partially inhibited by Mytelase (Figs 3 5) The commercial acetylcholinesterase preparation showed a diffuse band extending from 3 I to 5 It was strongest at both ends and the whole band was inhibited by Mytelase Heating for 30 min at 60°C caused disappearance of all esterase bands

Histochemical analysis

Pieces of postauricular and ear canal skin and of cholesteatoma epithelium were deep-frozen

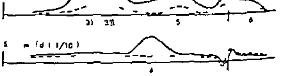


Fig 4 A pherogram of the electrophoretic run shown in Fig. 2 Eserine causes marked inhibition of fractions 3 I 3 II and 5 total inhibition of fraction 4

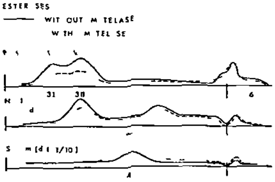


Fig 5 A pherogram of the electrophoretic run shown in Fig 3 Mytelase causes slight inhibition of fractions 3 I and 3 II.

and cryostat sections made for esterase staining The general pattern in all tissues was much the same the main esterase activity being evident in the transitional zone (Fig 6) It also appeared around the hair shaft and sebaceous canal in the postauricular skin and in histiocytes and cells lining small mucosal cysts in granulation tissue No activity was seen in the keratin layer In one child of 6 years with cholesteatoma large pools of esterase activity were seen subepithelially (Fig 7)

All control specimens without substrate were negative Similarly the sections kept for 1/2 hour in phosphate buffer at pH 7 8 without inhibitor showed a regular enzyme pattern The use of Eserine 10^{-} abolished about 80% of esterase activity and there was visible activity in concentrations higher than 10^{-4} and 10^{-3} Iso OMPA at 10^{-5} abolished only 10 to 20 % of esterase activity The concentration 10^{-4} was much more effective but some activity remained even after treatment with Iso-OMPA 10^{-3} Using BW284c51 in the concentration 10^{-5} some activity (about *10°*) disappeared The remaining activity remained unaltered in concentrations of 10^{-4} and 10^{-3} Combination of Eserine and Iso OMPA in the concentration 10^{-} abolished most of and 10^{-4} all of the visible esterase A similar result was obtained with eserine and BW284c51 With Iso-OMPA and BW284c51 combined in a concentration of 10^{-} only a small amount of esterase activity was abolished Increase of the concentration to 10^{-4} resulted in a significant further loss but a slight amount remained even when the concentration was increased to 10^{-3}



Fig 6 Esterase staining of cholesteatoma epithelium using α naphtylacetate as substrate The black brown esterase activity is seen in the whole transitional zone The keratin layer shows no acti ity (105)



Fig 7 Esterase staining of an active cholesteatoma membrane in a child Large pools of esterase activity are seen in submucosa in addition to the transitional zone staining (upper) Inhibition by a combination of BW284c51 and Iso-OMPA in a concentration of 10^{-4} M effected only partial disappearance of activity due to high esterase content (lower) Similar effect was seen with Eserine 10 M (×40)

COMMENT

In the epithelial specimens fraction 3 II consisting of cholinesterase and acetylcholinesterase and perhaps α_1 arylesterase and showing the mobility of α_1 globulin dominated in all samples Cholinesterase also formed the main part of fraction 3 I the whole of fraction 4 and part of fraction 5 whereas a weak acetylcholinesterase activity occurred in fraction 3 I and a stronger one in fraction 5 which corresponds well with test results using both the commercial preparation and nucleus caudatus homogenate Fraction 3 II in addition con

tained α_1 arylesterase which considering its relative weakness and inconsistency of occurrence in serum is very likely derived from the examined tissues and not from blood contamination Fraction 6 must be placed in the group of tissue esterase (Scheiffart et al 1966)

Cholinesterase was the dominating esterase in all epithelial specimens containing fractions which showed four different electrophoretic mobilities The next in order was acetylcholinesterase which appeared in 3 bands with no preference for any of the tissues examined Tissue esterases were stronger in cholesteatoma epithelium than in others The esterase pattern in epithelial tissues was clearly different from that in serum

A comparison with the histochemical tests confirms the main results of the electrophoretic analysis Electrophoretic separation of esterases was more informative since only by this means could the several different cholinesterase and acetylcholinesterase bands be disclosed The α_1 arylesterase and tissue esterase fractions must be relatively small as no visible esterase activity remained in tissue sections after inhibition with combinations of Eserine Iso OMPA and BW284c51

Even if the main series of cholesteatoma epithelium showed a pattern with only small differences as compared to skin the subepithelial esterase pools in active child cholesteatoma and the nearly missing activity in the adult cholesteatoma epithelium under considerable keratin pressure testifies to the part played by enzymes in epithelial control For the time being however the enzymatic action in the process of keratinization remains poorly understood

ZUSAMMENFASSUNG

Die Esterasen aus postaurikularer Haut und Gehorgangshaut Kolesteatomepithel und Granulationsgewebe wurden mit elektrophoretischen und histochemischen Methoden untersucht Die Esterasen aktivitat des Epithels wurde in Stratum granulosum lokalisiert Die Enzymverteilung in den Geweben war nicht dieselbe wie im Serum Im Epithel hatte Cholinesterase die grosste Aktivitat meistens mit α Globuline ab r auch mit α- und β_1 Globuline Eine Schwachere Aktivitat für Acetylcholinesterase wurde mit α und β Globulinen demonstriert Eine ganz schwache α Arylesterase aktivitat und eine starkere Gewebe-esteraseaktivitat mit γ Globuline wurden entdeckt Die elektrophoretische Methode war empfindlicher als die histochemische

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SODIUM ION IN THE STRIA VASCULARIS AND SPIRAL LIGAMENT

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Abstract Potassium pyroantimonate added to fixative solutions has been used to study tissue localization of sodium ions The resulting precipitate in the guinea pig stria vascularis and spiral ligament is distributed throughout the tissue The greatest concentration of the deposits is localized along the cellular side of the infolding plasma membranes of stria epithelium In the spiral ligament the dense concentration of the precipitate is found along the cellular side of spiral ligament connective tissue cells These findings suggest that sodium ions might be transported through the stria vascularis under the influence of ATP ase localized on the infolding plasma membrane of the stria epithelium but the role of the spiral ligament in the ion transport is not certain

The stria vascularis and its possible functions have intrigued anatomists since the development of the light microscope in the middle of 19th century Guild (1927) provided the modern impetus for the theory that the stria vascularis was involved in the secretion of endolymph In recent years ultramicroscopic studies have quickened interest in the possible function of the stria vascularis

The concentrations of potassium and sodium in endolymph approximate intracellular fluid while those of perilymph approximate cerebro spinal fluid and serum (Smith et al 1954 Citron et al 1956) The highly developed epithelial and vascular nature of the stria vascularis suggests a morphological model for maintenance of endolymph ion composition

The localization of *sodium* in the avian salt gland by means of an electron dense precipitate of sodium pyroantimonate was first used by Komnick (1962) Since then the ultrastructural method for the localization of sodium has been attempted in a variety of tissues by several authors (Kaye et al 1965 Hartmann 1966 Zadunaisky 1966 Kaye & Tice 1966 Spicer et al 1968 Edelhauser & Siegesmund 1968 Bulger 1969) Potassium pyroantimonate has been used to react specifically with sodium ions in biological fluids (Peters & van Slyke 1932) The principle of this method is the precipitation of sodium ion as pyroantimonate salt when in contact with potassium pyroantimonate The latter salt is *soluble in* water whereas the sodium one is not (Komnick & Komnick 1963)

The study herein reported applies this method in an attempt to localize the sodium ion in stria vascularis and the spiral ligament The preservation of the tissue fixed in the presence of the pyroantimonate reagent is not ideal when compared with the preservation of specimens prepared with the usual fixatives However the electron micrographic image is adequate to recognize the cytological relationships between the precipitate and fine structure of the stria vascularis and spiral ligament

MATERIAL AND METHODS

Adult male guinea pigs weighing about 350 g were used in this study Immediately after

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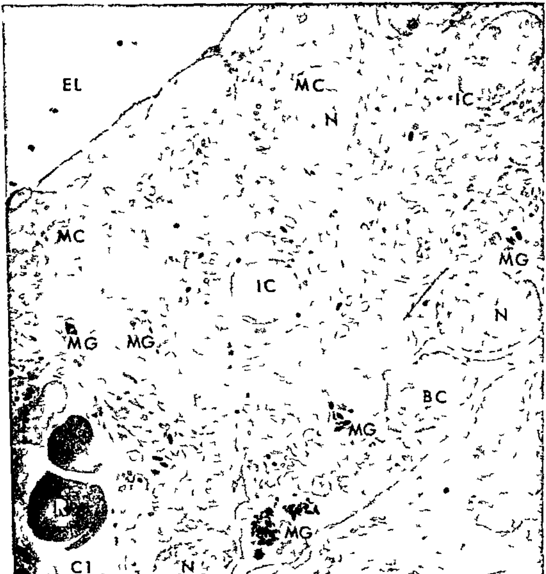


Fig 1 Electron micrograph of stria vascularis and spiral ligament from a normal guinea pig fixed in 1% OsO containing 2% potassium pyroantimonate Stria vascularis consists of marginal cells (*MC*) inter mediate cells (*IC*) and basal cells (*BC*) d with capillaries (*C1*) The spiral ligament nective ti sue cells (*CC*) and capill electron opaque precipitate throughout this organ *R* red blood granules *N* nucleus *EL*

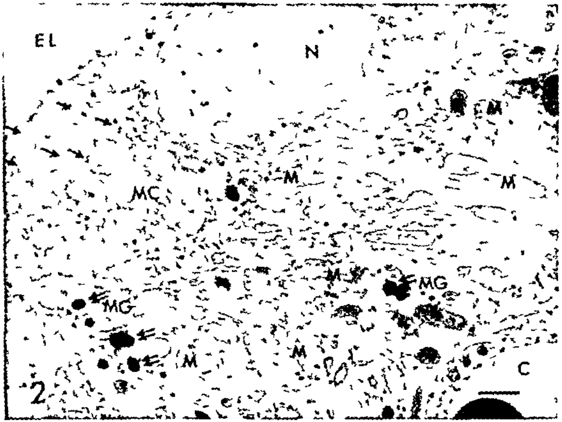


Fig 2 Electron micrograph showing endolymphatic surface of stria vascularis fixed in 1% OsO containing 2% potassium pyroantimonate The marginal cell (*MC*) shows precipitate in the nucl us (*N*) mitochondria (*M*) and small vesicles of apical cytoplasm (*arrow*) MG (*double arrows*) melanin gr nules *C* capillary *EL* endolymph × 10 000

oval the cochlea was opened and placed in 1% unbuffered osmium tetroxide at 4°C for one half hour It was subsequently immersed in osmium tetroxide potassium pyroantimonate solution for another half hour The latter fixative was prepared by following processes (Hartmann 1966) 0 5 g of osmium tetroxide was dissolved in 40 ml of a 2% aqueous solution of potassium pyroantimonate 0 5 to 1 0 ml of 0 1 N hydrochloric acid was added to the mixture the pH was adjusted to 7 6 with 0 01 N acetic acid and the volume made up to 50 ml with distilled water Specimens were dehydrated in graded acetone solutions and embedded in Epon 812 Because contrast in cell membranes remained low in these preparations some specimens were dehydrated for 15 min each in 50% and 70% acetone saturated with uranyl acetate Ultra thin sections were cut with diamond knives on an LKB ultratome and examined in an RCA EMU 3G electron microscope

RESULTS

The stria vascularis has a complicated cellular arrangement consisting of the three layers of epithelial cells resting upon the spiral ligament (Fig 1) The cells facing endolymph have a dense ground substance and are called marginal or dark cells The cells abutting the spiral ligament are basal cells The intermediate cells have a lighter cytoplasm and are situated between the marginal and basal cells Each of the cell layers is characterized by complicated cytoplasmic infoldings Capillaries are interspersed among the stria cells but are separated from them by double basement



Fig 3 Electron micrograph showing the infolding plasma membranes between marginal and intermediate cells of a guinea pig fixed in 1% OsO containing 2% potassium pyroantimonate The distribution of electron-opaque precipitate is spotty and is predominantly present along the cellular side of plasma membranes The precipitate appears in the matrix of mitochondria (M) *MG* melanin granules × 11 000

membranes The stria cells are in direct contact with the connective tissue of the spiral ligament without an interposing basement membrane The spiral ligament consists of connective tissue cells intercellular substance and capillaries

In the capillaries precipitate of sodium ion is seen within the lumen and in the endothelial cells (Fig 2) Occasionally pinocytotic vesicles of the endothelial cell contain the dense precipitate

The electron-opaque precipitate of what is probably sodium pyroantimonate appears more or less uniformly distributed throughout the cytoplasm and nucleus of the marginal cells (Figs 1 2) Precipitate is present in the mitochondria sometimes observed within the nuclei and frequently situated within small vesicles near the endolymphatic surface (Fig 2 arrow)

Sodium pyroantimonate precipitate is observed sparsely throughout the cytoplasm of the intermediate cells It is localized within the cisternae of endoplasmic reticulum and in the matrix of the mitochondria Little precipitate is found within the nucleus In the complicated infolding plasma membranes between marginal and intermediate cells electron-opaque granules are spotty and located along the cellular side of the cell membranes (Fig 3) Mitochondria in cytoplasmic extensions of both the marginal and intermediate cells also contain precipitate but less abundantly

The cells of the basal layer are elongated and have a clear cytoplasm They lie in a tightly packed layer between the light and dark cells and the spiral ligament Sodium pyro-



Fig 4 Electron micrograph showing the connection between stria vasculanis (*SV*) and spiral ligament (*SL*) *fixed in 1% OsO containing 2% potassium pyroantimonate* The distribution of sodium pyroantimonate shows a dense accumulation of precipitate in spiral ligament *BC* basal cell *CC* connective tissue *cell* ×9 000

antimonate precipitate is minimal in the basal cells and *localized within the cytoplasm* (Fig 4)

The elements of the spiral ligament are in direct contact with the basal cells without an intervening basement membrane The spiral ligament cells adopt an oval or somewhat irregular shape and possess a clear cytoplasm *containing few cell organelles Bundles of filaments* can be observed in the intercellular space The *sodium pyroantimonate* precipitate is localized within the cytoplasm of the spiral ligament cells especially within cisternae of the endoplasmic reticulum the mitochondria and the nuclei Furthermore a large number of electron-opaque particles are also found along the cellular side of the plasma membranes (*Fig 5*)

In the spiral ligament the capillary walls consist of endothelial cells and pericytes The sodium pyroantimonate precipitate is found within the cytoplasm of both (Fig 6) A great amount of precipitate is seen in the capillary lumen and electron-opaque particles are some*times found within the large pericapillary* spaces

DISCUSSION

Electron-opaque deposits are seen in the stria vascularis and spiral ligament after fixation in solutions containing 2% potassium pyroantimonate Deposits vary in shape and size but

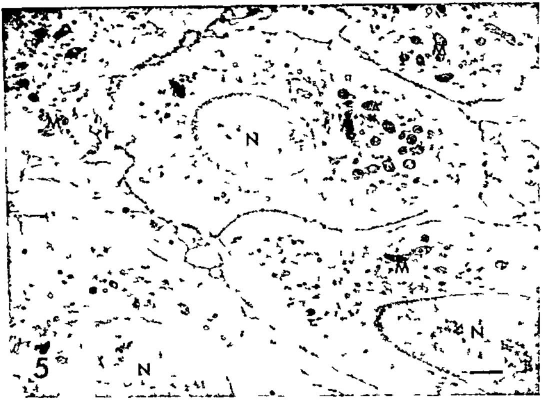


Fig 5 Electron micrograph showing the connective tissue cells of spiral ligament from guinea pig fixed in 1% OsO containing 2% potassium pyroantimonate Dense precipitate can be seen in the nucleus (N) and mitochondria (M) of spiral ligament A large amount of electron opaque precipitate is distributed along the cellular side of plasma membrane × 8 000

are generally consistent in any given structure The small ones are approximately 100 Å in diameter but deposits many times larger are seen The deposits in the cytoplasm of marginal intermediate and basal cells appear to be randomly distributed Most of this precipitate appears to be closely related to mitochondria endoplasmic reticulum and nuclei The greatest concentration of precipitate in the stria is on the infolding plasma membranes between marginal and intermediate cells These deposits are localized along the cellular side of the cell membranes In spiral ligament cells the precipitates are found in the cisternae of endoplasmic reticulum and mitochondria and dense concentration of the deposits is seen along the cellular side of cytoplasmic membranes

The application of potassium pyroantimonate during fixation provide a reproducible pattern of precipitation in the stria vascularis and spiral ligament Combined morphological and biochemical or histochemical studies may be helpful concerning the functional significance particularly of fluid transport in the stria vascularis and spiral ligament Based on experimental studies Kaye et al (1965) suggested the possible relationship between ATP ase reaction and sodium pyroantimonate precipitate They reported that an electron opaque precipitate located along the cellular side of rabbit cornea epithelium might present the site of a saturated high affinity Na+ carrier from the effect of ouabain on sodium pyroantimonate deposits As a hypothesis of the relationship between sodium pyroantimonate deposit and ATP ase activity Kaye et al (1966) suggested that the increase of sodium pyroantimonate precipitate after exposure of

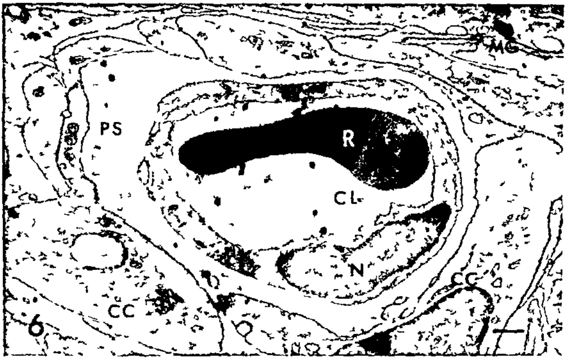


Fig 6 Electron micrograph of a spiral ligament capillary fixed in 1 OsO containing 2% potassium pyroantimonate The electron opaque precipitate can be seen in the capillary lumen (*CL*) and within the large pericapillary space (*PS*) *R* red blood cell *N* nucleus *CC* spiral ligament cells *MG* melanin granules × 8 000

the cornea to ouabain may be a direct result of ATP ase inhibition

Recently Nakai & Hilding (1966) reported he distribution of ATP ase activity in the ria vascularis and spiral ligament which howed the reaction product on the cell membrane and basement membrane of blood vessels They also stated that the infolding plasma membranes in the stria vascularis were strongly active The precise localization of ATP ase reaction and dense concentration of sodium pyroantimonate on opposite sides of the infolding plasma membranes of the strial epithelium supports the thesis of a possible role in fluid transport Although the greatest concentration of precipitate is found along the cellular side of the spiral ligament cells a biochemical study showed that ATP ase is primarily localized in the stria vascularis and little if any is present in the spiral ligament (Iinuma 1967) Evidence supporting the relationship between ATP ase reaction and sodium pyroantimonate deposits in the spiral ligament cells are not certain in this study

ZUSAMMENFASSUNG

Die Lokalisation von Natriumionen in der Stria vascularis und des Ligamentum spirale wurde mit Komnickschen Techniken in Verbindung von analytisch chemischen und elektronenmikroskopischen Praparationsmethoden studiert Eine hohere intrazellulare Natriumionen Konzentration wurde an den komplizierten fingerformigen Fortsatzen der Stria vascularis und an den Zellen von Ligamentum spiral gefunden Die Bedeutung der Resultate wird diskutiert

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SISI TEST AND ADAPTATION

I Subjects with Normal Hearing

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Abstract The effect of the adaptation of the ear upon the SISI test was studied in 26 normally hearing persons The average adaptation was found to be 4 2 dB at 250 Hz, 7 5 dB at 500 Hz and about 10 dB at higher frequencies after 3 min sustained stimulation In the preadaptation test the average SISI test values ranged from 22 5% to 36 2 In the post adaptation SISI test the average figures ranged from 30 8 to 48 7% The dispersion of preadaptation SISI values was very large which makes evaluation of the results difficult in a normal material 15% showing positive results in Jerger s classification Adaptation increased the positive values at higher frequencies but changes to both directions occurred

Changes in the differential sensitivity to inten (DLI) were thought to provide a new and test in audiological differential diagnosis 20 years ago Since the study of Hirsh et al (1954) however interest in this test in its conventional form has waned because no clear separation could be effected between various clinical groups On the basis of these findings Jerger et al (1959) designed a new test using sustained stimulation to replace the conventional DLI tests In this test the subject attempts to distinguish 1 dB pulses of 200 msec duration presented at 5 sec intervals in a continuous 20 dB sensation level tone The number of the recognized pulses (per cent) was termed as Short Increment Sensitivity Index (SISI)

Jerger and his associates later reported (1961 1962) results of the SISI test in normal hearing and in conductive cochlear and retrocochlear deafness It was concluded (1961) that values above 55% were positive (i e indicative of abnormally keen differential sensitivity) those between 20–55% questionable, and those below 20% negative The test–retest trials by Jerger (1962) showed a slight practice effect the performance tends to be somewhat better on the retest The largest test–retest difference occurred at 4 000 Hz (12 3%) The standard error of the measurement was found to be 20% at 250 and 1 000 Hz and 13% at 4 000 Hz

Several other variables of the SISI procedure in normal material have been investigated e g changes in the pulse intensity in the sensation level and in the number of pulses presented Yantis & Decker (1964) using increments of 1 2 3 and 4 dB noted that the percentages grew as the tone pulses became larger a finding that can be expected Hanley & Utting (1965) using increments of 1 0 75 and 0 5 dB in 48 subjects obtained a mean score of 42% for 1 dB steps and 15 for increments of 0 75 dB They felt that clinical separation with 1 dB steps was not feasible but that 0 75 dB should be used Blegvad (1966) using 40 subjects noted an increase in the SISI values with the frequency increasing from 250 to 4 000 Hz when the sensation levels were 10 and 20 dB but at 40 dB the scores were grossly independent of frequency Since many materials had large positive values in normal ears Rubinstein

et al (1970) suggested that only average values of the four tested frequencies (500 1 000 2 000 and 4 000 Hz) should be used

Blegvad & Terkildsen (1966) noted that increased percentages for SISI were obtained from the test ear if the contralateral ear was masked This was particularly true of the higher frequencies This finding was confirmed by Osterhammel et al (1970) who used direct measurements of evoked cortical responses to SISI type stimuli

From the results of suprathreshold adaptation tests (Palva & Palva 1963 Karja 1968) it is known that even at 20 dB sensation level a marked adaptation can develop quite rapidly even in normal ears In the SISI procedure the sustained host tone lasting for 1 min 40 sec must also be subject to similar adaptation This study has been undertaken to determine the effect of this suprathreshold adaptation upon the SISI scores in normally hearing subjects

MATERIAL AND METHODS

The material consisted of 26 persons between 21–30 years (average age 25 5 years) with normal hearing between 250 and 6 000 Hz measured with the Madsen OB 60 audiometer Most of the subjects were either students or graduates from the university there were 10 men and 16 women

The subject was seated in a sound isolated room while the equipment and the investigator were in an adjoining sound treated room The background noise measured with the Precision Sound Level Meter Type 2203 Bruel & Kjaer was less than 44 dB (overall) at the place of the subject Individual hearing thresholds and adaptation measurements were made with a self recording Grason Stadler Type E 800 audiometer using intensity change of 0 25 dB steps at a rate of 2 1 dB/sec

For interrupted tones the pulse duration was 200 msec with a rise and decay time of 25 msec and an on-off ratio of 1 1 The second channel of the Grason Stadler audiometer was connected with a Philips GM 238 Tone Generator the

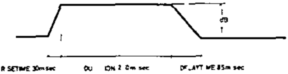


Fig 1 The dimensions of the tone pulse used in the short increment sensitivity test

signals being measured and attenuated by Grason Stadler voltmeter and attenuators The equipment was calibrated at the beginning of the test and several times during the course of the tests

A Madsen SISI adaptor giving tone pulses of 5 and 1 dB intensity with a duration of 200 msec was used The interval between the tone pulses was changed to 3 5 sec but in the actual test they were presented to the subject at irregular intervals first manually later regulated by an automatic timer The total duration of the test nevertheless was kept within the normal time limits suggested by Jerger viz. 20 × 5 sec or 1 min 40 sec This test arrangement was considered necessary in order to prevent any automatism that the subject might develop if the pulses were to follow at regular intervals The duration intensity and form of the tone pulses were checked at 1 month intervals The rise time was 30 msec duration 240 msec and decay time 85 msec when the intensity was 1 dB (Fig 1)

Fig 2 shows the block diagram of the apparatus The subject used the subject switch to regulate the intensities of the tones presented during the adaptation measurement. A similar method was used in the binaural balance tests the recording of the excursions being made by the channel I recorder in the usual way

The tests were started by determining the air conduction thresholds in both ears by using tones of 1 000 2 000 4 000 6 000 1 000 500 and 250 Hz in that order A pulsed tone with the fixed frequency technique was employed and the test subjects pressed the button when they heard the tone and released it as soon as the tone disappeared

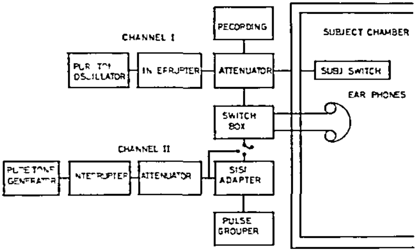


Fig 2 Block diagram of the testing equipment.

The subject was acquainted with the SISI test by presenting a few 5 dB tone pulses as an example The test ear was then given a 20 dB sensation level tone through channel II and the SISI test was made with 1 dB tone pulses as described above at the frequencies concerned The test subject informed the investigator by light signals whether or not he heard the tone pulses After this test a rest period of 6 min was allowed to let the possible adaptation disappear

Before the adaptation test was started the test ear was given a 20 dB SL pulsed tone through channel II while the subject made a balancing record with the control ear through channel I The pulses were simultaneous in both ears and this recording gave the estimate of the starting level for adaptation at any tested frequency

The actual adaptation measurement was started by making the tone continuous in the test ear From this point onwards the subject used the subject switch to regulate the pulsed control tone coming through channel I into the contralateral control ear to have the same loudness as the test tone In this way the adaptation recording was made during a period of 3 min Without interrupting the continuous tone in the test ear the SISI test was performed in the test ear by using tone pulses of 1 dB intensity at all the frequencies mentioned The first test with 1 000 Hz tone was always given as a practice run and was not included in the results

For statistical treatment the amount of adaptation was measured from the recordings at the points of 0 15 30 45 60 90 120 150 and 180 sec intervals the reference value being the preadaptation balancing level The SISI values were obtained directly in percentages in the audiometric examination The data were handled at the Computer Centre of Oulu University After determining the averages and their standard deviations the significance of the results was measured by using Student s *t* test with a significance level of $p < 0\ 01$

RESULTS

Table I presents the average hearing thresholds and their standard deviations In addition the

Table I *Average hearing thresholds (re 0 0002 dyn/cm) and balance levels before adaptation (SL)*

Frequency (Hz)	Hearing thresholds				Balance levels	
	Adapting ear (dB)		Control ear (dB)		Control ear (dB)	
	Mean	S D	Mean	S D	Mean	S.D
250	28 5	3 6	28 0	4 5	17 4	5.3
500	10 9	3 0	12.1	3 9	18 8	4 1
1 000	7 2	3 6	5 0	3 8	18 6	3 8
2 000	8 8	2.8	7 3	3 9	18.2	5 4
4 000	8 1	4 3	7 7	5 1	18 1	5 0
6 000	17 8	5.2	19 8	4 0	13 4	4 9

Table II *Mean results of perstimulatory suprathreshold adaptation (dB) for continuous pure tones during 3 min at 20 dB SL*

Freq (Hz)	Time sec 15		30		45		60		90		120		150		180	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
250	1 3	1 9	2 6	3 2	2 7	4 3	2 8	4 2	3 3	4 5	3 3	4 5	3 6	4 8	4 2	4 4
500	1 0	2 0	3 3	3 3	5 0	4 3	5 6	4 3	6 3	5 3	6 7	5 4	6 9	5 5	7 5	5 5
1 000	3 3	2 8	6 2	3 9	7 3	4 5	8 2	4 6	8 8	4 8	9 2	5 0	9 8	4 7	10 3	4 6
2 000	3 5	2 5	7 1	3 3	8 7	5 0	10 1	5 1	11 3	6 8	12 3	6 6	12 3	6 6	13 1	6 5
4 000	3 4	3 1	7 6	4 5	9 6	4 4	10 5	4 9	11 1	4 5	11 7	4 4	11 9	5 1	12 9	4 9
6 000	3 3	2 5	6 0	3 6	7 2	4 0	7 6	4 4	8 1	4 7	8 7	4 8	8 2	4 1	9 9	5 0

table shows the balancing levels between the adapting and control ear before the adaptation test. All average balancing values are slightly below 20 dB level the most notable deviation begins at 6 000 Hz (13 4 dB)

Table II shows the average amount of adaptation at different frequencies with their standard deviations as a function of time The amount of adaptation is lowest at 250 and 500 Hz, being 4 2 and 7 5 dB respectively while at the higher frequencies the measured adaptation generally slightly exceeds 10 dB These results are shown graphically in Fig 3 In the average curves the amount of adaptation has reached its main level in one minute s time and the changes thereafter are slight

Comparison between the practice test at 1 000 Hz as the starting frequency and the test made later at the same frequency showed slightly lower (2 3 dB) balancing level in the ordinary test Similarly in adaptation tests practice made the adaptation drop by 3 5 dB in 3 min at 1 000 Hz runs

The average values for the SISI test before and after 3 min 20 dB SL adaptation are shown in Table III The preadaptation values range from 22 5 to 36 2° while the postadaptation values range from 30 8 to 48 7 The averages thus show a tendency to higher percentages but the very large range of observations gives no statistical significance to the average results This range of results is seen in Figs 4 and 5 which present the individual data at the tested frequencies before and after adaptation

Examination of the individual data on adaptation showed that in 15 test subjects adaptation reached its plateau within 1 min whereas in 10 subjects adaptation increased slowly during the whole test time reaching its plateau at 3 min One subject showed no noticeable adaptation at any frequency

Breaking down the average SISI data individually by using Jerger s limits showed that the negative and questionable groups were the largest but that about 15 showed positive results This distribution is shown relative to the different frequencies in Table IV It

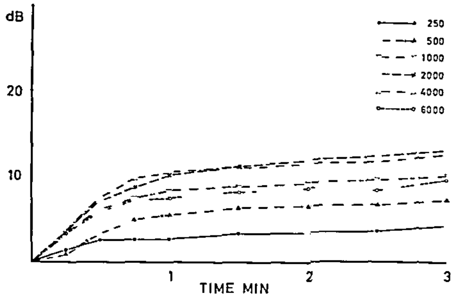


Fig 3 Perstimulatory suprathreshold adaptation as a function of frequenc during 3 min testing wit sustained tones at 20 dB SL.

that th. larger scores increase in the postadaptation group particularly at the higher frequencies

The average SISI data were also grouped anew according to the fast or slow development of adaptation This did not reveal any significant differences between the two groups

The individual changes in the SISI score at different frequencies are seen in Fig 6 Even though the increase in the SISI scores is more frequent in the postadaptation values there are many ears of which the reverse is true These changes are also summarized in Table V

DISCUSSION

The normal scores for the SISI test were first defined to be between 0–20 (Jerger 1959) and in clinical material values of 0–15 % were held as negative 20–55 % as doubtful and 60–100 % as positive (1961) Normal values by Yantis & Decker (1964) were also generally under 20 % with the exception of 4 000 Hz at which frequency individual values went up to 85 % 1/3 of the material showing values over 60 % The average preadaptation figures in the present study (from 22 5 % to 36 2 %) are clearly higher than those referred to above and closer to the data of Hanley & Utting (1965) who reported a percentage of 42 4 at 4 000 Hz The average results 6–16 of Blegvad (1966) are somewhat lower than the

Table III *SISI scores before and after 3 min 20 dB SL adaptation*

Frequency (Hz)	Before adaptation Mean	S D	After adaptation Mean	S D
250	28 8	25 0	31 2	27 0
500	29 8	25.2	30 8	23 9
1 000	31 1	24 7	33 7	29 3
2 000	22.5	24 1	40 4	28 0
4 000	36.2	24 0	48 7	25.2
6 000	27 9	19.2	38 5	24 7

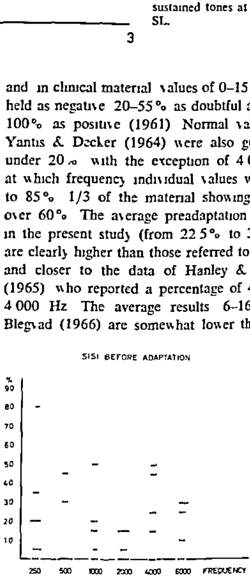


Fig 4 Individual SISI scores at various frequencies in the preadaptation test

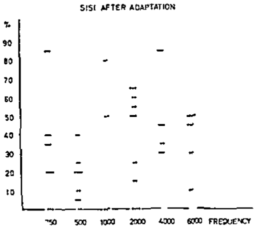


Fig 5 Individual SISI scores at various frequencies in the postadaptation test

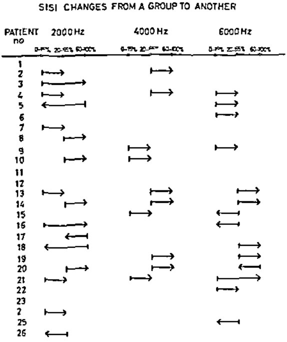


Fig 6 Changes in the SISI scores from one group (Jerger) to another after adaptation at 250 to 1 000 Hz and 2 000 to 6 000 Hz. The arrows show the direction and magnitude of the change

present data The results of Rubinstein (Rubinstein et al 1970) show a similar large range of observation but somewhat smaller average values

The SISI scores obtained in normally hearing persons show a discouragingly large dispersion and support the suggestion of Yantis & Decker (1964) that the limit of scores considered positive should be raised from 60 to 80 %. The present data also support the view of Hanley & Utting (1964) that the test should be made with increments of less than 1 dB intensity to increase the sensitivity of the test

The preadaptation balance levels were in the present study lower than the 20 dB reference level in the control ear at all frequencies The differences were not large except at 6 000 Hz but were all opposite in direction to those reported by Karja (1968) The reason for these deviating data remains obscure The development of adaptation and the form of the average curves agrees well with the data of Karja the higher frequencies showing clearly more adaptation than the low frequencies The small observed value for adaptation at 6 000 Hz can apparently be related to the low balancing level and correspondingly to a slightly lower sensation level during the test.

The three basic types in the development of adaptation as a function of time which were

Table IV *SISI values in various groups before and after adaptation*

Frequency (Hz)	Before adaptation			After adaptation		
	0–15	20–55	60–100	0–15	20–55	60–100
250	9	13	4	9	12	5
500	10	12	4	8	14	4
1 000	9	10	4	9	9	5
2 000	15	8	3	7	11	8
4 000	7	15	4	3	13	10
6 000	9	16	1	6	15	5

Table V *Changes of SISI percentages from one group to another after adaptation*
p = positive n = negative q = questionable

Frequency (Hz)	Unchanged ( )	p n ( )	p q ( )	n p ( )	n q ( )	q n ( )	q p ( )	Total ( )
250	62	8	0	0	11	8	11	100
500	76	0	8	0	8	0	8	100
1 000	65	0	4	0	13	9	9	100
2 000	39	8	4	8	22	4	15	100
4 000	62	0	0	0	15	0	23	100
6 000	46	0	4	4	19	12	15	100

observed by Karja were confirmed in this study In this series rapid growth of adaptation oc curred in 58% slow growth in 38%, and no adaptation at all was found in 4° The cor responding percentages by Karja were 47 50 and 5% respectively

The main question i e how adaptation af fects the SISI values is partly obscured if only average values are considered The average re sults tend to show elevated percentages in the post adaptation test but the large dispersion of the results makes statistical evaluation nonre warding When the data are broken down individually all the different types of behaviour in normal ears can be seen in one group the adaptation test has no effect upon the observed SISI values while in the other group there is a clear tendency for the SISI values to become either larger or smaller as a result of adapta tion

In the postadaptation SISI test the adapta tion had reduced the loudness level in the test generally by more than 10 dB at frequencies above 1 000 Hz This as such had no effect upon the percentual SISI values It agrees with the contention of Jerger (1959) that use of a continuous tone in the SISI test would not introduce factors that would unfavourably af fect the results In the present normal material the test tone did not disappear totally in any case and the pulses were always heard against the tonal background In pathological ears the tone may sometimes wane out entirely in spite of this the tone pulses were still heard in some diagnostic groups (Owens 1965 Hughes 1968) The behaviour of ears with impaired hearing after preliminary adaptation in the SISI test is presently under study

ZUSAMMENFASSUNG

Die Wirkung der Adaptation auf den SISI Test wurde bei 26 Ohren mit normalem Gehor untersucht Es wurde festgestellt dass Adaptation nach 3 min Sti mulation durchschnittlich 4 2 dB bei 250 Hz 7,5 dB bei 500 Hz und ungefahr 10 dB bei hoheren Fre quenzen betrug Bei dem Praadaptations SISI Test *erstreckten sich die durchschnittlichen SISI Test Werte* von 22 5% bis 36 2 % Bei dem Postadaptations SISI Test waren diese Werte von 30 8% bis 48 7 Die Streuung der Praadaptations SISI Werte war sehr gross und machte die Bestimmung des Ergebnisses bei normalem Material schwierig wenn 15 schon posi tive (pathologische) Ergebnisse anzeigten (nach der Klassifikation von Jerger) Die Adaptation vermehrte die positiven Werte bei hoheren Frequenzen aber Abweichungen nach beiden Richtungen traten auf

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DIRECTIONAL AUDIOMETRY

II The Influence of Azimuth on the Perception of Speech

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Abstract In 30 persons with normal hearing a previously described apparatus (Tonning 1970) was used for free field d terminations of the intelligibility of speech in relation to the azimuth of the source Directional Threshold of Intelligibility (DTI) was recorded both with and without background noise Two loudspeakers the one producing sp ech and the other producing white noise could be rotated independently in the horizontal plane around the test person p r mitting an analysis of the depend ncy of the DTI upon the positions of th speech and the noise source The assumption is made that DTI is dependant on age With the help of electronic computer analysis th material collected during the examinations was used to construct a standard diagram showing the DTI values in persons with normal hearing with and without background noise

Experiments demonstrating that the intensity and character of sound vary as a function of the angle of incidence at the ears have been published by Firestone (1930) Rosenberg & Slavinsky (1940) Nordlund (1962) Nordlund & Liden (1963) and Harrison & Downey (1970) From these experiments it may be assumed that the threshold of intelligibility of speech would probably also vary with the angle of incidence of the sound at the ears Hirsh (1950) made use of loudspeakers in free field by investigating the threshold of speech intelligibility against background noise He found that the threshold depended on the interaural phase angles of the speech and noise stimuli The highest threshold for subjects with binaural normal hearing occurred when the signal and noise loudspeakers had coincident positions Nordlund & Fritzell (1963) spectrographically analysed the qualitative variations in speech as a function of the azimuth of the source They demonstrated that the information reaching the ear turned toward the sound source was markedly greater than that reaching the ear turned away from the sound source These variations were greatest for frequencies above 2 000 Hz

MATERIAL AND METHODS

Throughout the following the dB reference level is 0 0002 dyne per square centimetre The experimental apparatus and the persons examined were the same as described by Tonning (1970) Loudspeaker *h*1 was used to reproduce speech while loudspeaker *h*2 reproduced white noise with an intensity of 65 dB at the point corresponding to the centre of the tested subject s head

Thirty persons with normal hearing were used as test material The Directional Threshold of Intelligibility (DTI) was recorded without background noise with the loudspeaker *h*1 in four different positions (0° = in front 90 = on the right side 180° = behind 270° = on the left side of the person tested) Then experiment was made in the same way with background noise (65 dB white noise) for each of the four positions of the loudspeaker *h*1 reproducing speech four positions (0

90 180 and 270) of the white noise source (loudspeaker $h2$) were used giving 16 different combinations of the loudspeakers $h1$ and $h2$

During preliminary free field examinations the threshold of speech intelligibility was examined with falling sound intensities and the point where the subject could perceive and repeat 50 % of the test words correctly was recorded In each person tested it was necessary to repeat the procedure 20 times—four tests without background noise and 16 tests with noise This resulted in so much fatigue in the test person and in such great dispersion of the figures recorded that it became necessary to abandon this method Instead it was decided that the level of DTI should be defined as the highest intensity level at which the subject could no longer understand and repeat the test words when they were presented with falling intensity During the examinations movements of the head were not allowed

The first words were presented with sufficient intensity to ensure that the subject could repeat them without difficulty The following words were then reproduced with falling intensity in steps of 1 dB per word When the intensity level at which the subject could no longer perceive and repeat a word correctly was reached the sound intensity was held constant while three words more were presented If these words then were repeated incorrectly the indication of the attenuator was recorded If any of these words were repeated correctly the intensity was lowered further in 1 dB steps until 4 words could not be perceived and repeated correctly Presenting the test words with falling intensity the DTI is defined as the highest intensity level in dB re 0 0002 dyne per square centimetre at which the person tested can *not* perceive and repeat correctly 4 consecutive words The attenuator was calibrated in dB measured corresponding to the centre of the test subject s head This calibration was made without any test person in the sound field In the experiments described here we made use of the same phonetically balanced words as are used by the Danish State Hearing Center for Speech Audiometry

Table I *The DTI values for 30 subjects with normal hearing recorded without white noise for each position of the loudspeaker expressed by* $\overline{DTI}$ *= Mean of DTI for the 30 persons examined* *s = Standard Deviation*

	Position of the signal loudspeaker			
	Front	Right	Behind	Left
$\overline{DTI}$	15 6 dB	13 8 dB	16 0 dB	13 8 dB
s	1 63	1 77	1 46	1 98

DTI without noise

The DTI values were recorded without white noise one value for each person for each of the four positions of the signal loudspeaker In Table I the mean of DTI for the 30 persons (= $\overline{DTI}$) together with the standard deviation (= s) is tabulated for each of these positions

We assume the results to be independent and normally distributed for a given position P_1 with expected values E_p and with the same variance Rank ordered analysis of variance level of significance 0 05 was used No significant difference was found between the $\overline{DTI}$ values recorded with the speaker placed directly in front of or directly behind the subjects Likewise no significant difference was found between the $\overline{DTI}$ values with the loudspeaker directed at the right or left ear There was however significant difference between the values of $\overline{DTI}$ found with the signal loudspeaker in the positions right–left compared with the values found in the positions front–behind the highest $\overline{DTI}$ thresholds were found when the loudspeaker was placed directly in front of or behind the subject The lowest thresholds were found with the loudspeaker aiming directly at the right or the left ear

We wanted to find out whether irregularities in the acoustical properties of the test room affected the DTI For this purpose 5 of the persons were examined twice by the second examination the room was rotated 90° to the right around the subjects tested For each of the 4 positions of the signal source related to the person tested the hypothesis of being no difference between the DTI found by the first and second examination was not rejected (Wilcoxon test was used level of significance 0 05) However the small sample used in this test and the problems of the measurements of DTI in general can only give us a weak notion to what extent the room factor affects our experiments

(Statistics in this article According to Dixon & Massey 1957 Siegel 1956 Electronic computer was used the programmes being taken from IBM s System/360 Scientific Subroutine Package (360A CM-03X) Version III 1968)

DTI in noise

In this experimental series DTI was determined during simultaneous stimulation with white noise The signal loudspeaker was placed in each of the previously mentioned four positions in turn For each of these positions the noise loudspeaker was also placed in the same four positions resulting in a total of 16 different test combinations The results are tabulated in Table II where for each combination of the loudspeakers the mean value of the DTI (= $\overline{DTI}$) and the standard deviation are recorded

We assume the DTI values recorded independent and normally distributed for a given position P_i with expected values E_p and with the same variance for all of the listening conditions in noise Using rank ordered analysis of variance level of significance 0 05 (Sverdrup 1964) we get results as illustrated in Fig 1 where the different loudspeaker positions are arranged according to their numerical $\overline{DTI}$ values

From Fig 1 it can be seen that the highest

Table II *The DTI values for 30 subjects with normal hearing recorded against background noise for each position of the loudspeakers expressed by* $\overline{DTI}$ *= Mean of DTI for the 30 persons examined s = Standard Deviation*

Position of signal loudspeaker		Position of noise loudspeaker			
		Front	Right	Behind	Left
Front	$\overline{DTI}$	51 0 dB	45 3 dB	49 4 dB	45 1 dB
	s	1 58	1 90	1 77	2 07
Right	$\overline{DTI}$	43 6 dB	50 8 dB	43 2 dB	43 4 dB
	s	2 25	1 65	2 37	2 10
Behind	$\overline{DTI}$	50 0 dB	46 2 dB	50 5 dB	46 2 dB
	s	1 87	1 85	1 89	1 86
Left	$\overline{DTI}$	43 5 dB	43 4 dB	42 8 dB	50 8 dB
	s	2 06	1 87	2 47	1 75

(poorest) $\overline{DTI}$ values are found with both loudspeakers in the same position and when both loudspeakers are positioned coaxially in front–behind The lowest (best) $\overline{DTI}$ values are found when the signal loudspeaker is aiming at the right or the left ear while the noise loudspeaker is behind the examined person These results are in agreement with those of Hirsh (1950) For the other experimental listening conditions we find $\overline{DTI}$ values lying between these outer points

DTI with the signal loudspeaker in the lateral position

Licklider (1948) showed that intelligibility scores in binaural noise were as much as 12 2% higher when the speech was delivered to right ears than when presented to left ears Weston et al (1965) found only a side difference of 2 1% whilst Dirks & Carterette (1966) only could find a side difference of 1 8 Study of our collected DTI values has not revealed any significant difference between the values recorded with the signal loudspeaker aiming at the right ear as compared with those found with the signal loudspeaker aiming at the left ear (Test used Two samples Student s T test level of significance 0 05)

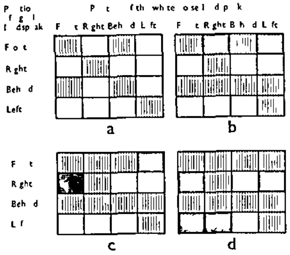


Fig 1 Rank ordered analysis of variance level of significance 0 05 Hatched squares indicate that the DTI values for the corresponding loudsp aker positions are significantly different from those of the white squares The DTI values of the dark squares are not significantly different from the values of eith r the hatched or the white squares White squares represent the lowest (best) DTI values

DTI related to age

The 30 persons examined were from 19 to 52 years old The average age was 26 7 years and the median age was 23 5 years The 30 persons were divided into two groups of 15 In the one group none was older than 23 years the average age was 20 5 years and the median age 20 years In the other group none was younger than 24 years the average age was 32 9 years and the median age was 31 years For all the loudspeaker positions the hypothesis of equal DTI values for the two groups was rejected (Test used Two samples one sided Student s T test level of significance 0 05) For the younger group the mean DTI was 14 7 dB for the older group the mean DTI was 16 1 dB $s = 1\ 3$ From this for people with identical pure tone audiograms we could expect the DTI to be higher for the older than for the younger persons However this dependency of the DTI on age is so small that a factor for correction was not found necessary for our purpose

Graphic presentation of the results

Due to the facts that the numerical value of DTI depends on the presence or absence of background noise and that the value further more depends on the relative position of the signal loudspeaker the noise loudspeaker and the subject s head it will not be possible to provide a standard diagram where a standard DTI could be given with one single value for all the possible relative positions Our observed DTI values could be plotted in a coordinate system where DTI recorded without noise was plotted as the abscissa and the DTI values for the same position of the signal loud speaker and with the noise loudspeaker in the four positions was plotted as the ordinate

A scattergram developed in this way would show that our measured DTI values clumped together in some way but it would be difficult to define the border of the area The prime intention with this examination of the persons with normal hearing was to present the results in a way permitting the DTI values of these persons to be used as a basis of comparison when later on evaluating the hearing in persons with hearing loss

Through electronic computer analysis of the records a coordinate system was constructed and found useful for this purpose the abcissa axis indicates values for DTI without noise The position of the signal loud speaker being the same the ordinate axis indicates values of DTI in noise divided by the corresponding DTI without noise i e the DTI value plotted as the abscissa Using electronic computer to plot experimental results in 16 above mentioned coordinate systems one for each combination of speakers points were produced which were lying in 16 bands If the highest and lowest DTI value without noise for each of these bands are plotted as the abscissa for the corresponding band lines through these points parallel to the ordinate cut these bands The results are 16 parallelograms The differences between these parallelograms are small The 16 parallelograms

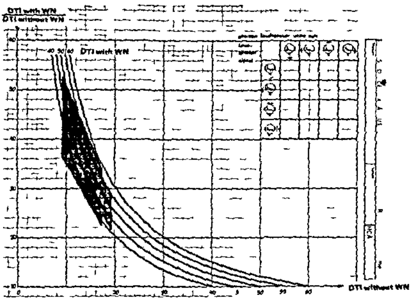


Fig 2 Coordinate system where the shaded polygon covers 95 of the DTI values for 30 persons with normal hearing examined For further explanation see text

present DTI values for different experimental conditions but the figures partially cover each other We therefore limit the area to cover 95% of the DTI values recorded The polygon thus developed is shown on Fig 2

In order to make the diagram more instructive and to avoid the trouble of calculating the fraction

$$\frac{\text{DTI with noise}}{\text{DTI without noise}}$$

five line segments are drawn plotting some of the most common values of DTI in noise namely DTI = 40 45 50 55 and 60 dB

The diagram is then used in the following manner

1 The value corresponding to an observed DTI without noise is found on the abscissa axis

2 Plot a point where this value s vertical is cutting the line segment of the corresponding observed DTI in noise interpolating if necessary

The position of a point in the coordinate system provides the following information

(a) DTI related to the thresholds of the normally hearing standard group If the point is falling inside the polygon the DTI does not differ from the thresholds of the standard group

(b) DTI without noise The further to the left the point is located in the coordinate system the better the DTI without noise If a line through the point normal to the abscissa is cutting the area of the standard persons the DTI without noise does not differ from the thresholds of the standard group

(c) DTI in noise The lower the value of the iso-line through the point (this iso line not necessarily drawn on the graph) the less is the perception of speech disturbed by background noise If the iso line through the point is cutting the polygon, it is an indication that the individual s perception of speech in noise under these actual conditions is corresponding to the values of the standard group

The location of a point in the coordinate system thus informs us about the subject s perception of speech with and without background noise Additionally it provides an opportunity to relate the individual s abilities to those of a standard group with normal hearing It should be mentioned that in the upper right corner of the coordinate system there is

a figure where the examiner can indicate the actual positions of the loudspeakers in each case

ZUSAMMENFASSUNG

Bei 30 Personen mit normalem Horvermogen wurde ein fruher beschrieb ner Apparat (Tonning 1970) fur frei Feldbestimmungen von Sprechverstandlich keit im Verhaltnis zum Azimut der Quelle benutzt Die gerichtete Verstandlichkeitsschwelle (DTI) wurde sowohl mit als auch ohne Hintergrundsgerausch re gistriert Zwei Lautsprecher von denen der eine Worte und der andere weisses Rauschen produzier ten konnten unabhangig von einander im Horizontal plan um die Test Person rotiert werden. Der Test ermoglichte eine Analyse der Abhangigkeit der Ver standlichkeitsschwelle (DTI) von den Positionen der Sprech und Gerauschquelle Es wird angenommen dass DTI vom Lebensalter abhangig ist. Mit Hilfe einer elektronisch n Computer Analyse wurde das in den Tests gesammelte Material zur Aufstellung eines Standard Diagrammes benutzt aus welchem die DTI Werte b i Personen mit normalem Gehor mit oder ohne Hintergrundsgerausche hervorgehen

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INCUS FIXATION DUE TO CONGENITAL ANOMALY OF CHORDA TYMPANI

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From the Department of Otolaryngology Tel Hashomer Government Hospital Tel Hashomer Israel

(Received April 15 1971)

Abstract A case of a rare anomaly of chorda tympani nerve is reported It differs from the known and functionally unimportant variations In this case a bony sleeve" of the nerve caused fixation of the incus, thus participating in the conductive hearing loss

The middle ear structures are often subject to isolated anomalies and malformations Several of these are asymptomatic some even minor may cause hearing impairment of varying severity (Henner & Buckingham 1956) Most of these variations are well known (Fowler 1951 Hough 1958 1963 Ombre 1959) Hough (1958) feels that many of them have been observed but not

We report here a case where a bony sleeve of chorda tympani nerve caused fixation of the incus The anomaly was revealed together with an ankylosis of the stapes footplate at an explorative tympanotomy for a non progressive conductive hearing loss with intact tympanic membrane

Case Report

A 16-year-old boy was seen in June 1970 because of bilateral hearing impairment This was first noticed at the age of six when following scarlet fever the patient had an acute otitis media in the left ear In spite of treatment the condition of the ear worsened and hearing progressively deteriorated Eventually at the age of 9 1/ because of an acute mastoiditis a radical mastoidectomy was performed

On examination the nose nasopharynx throat and larynx were healthy the eustachian tubes permeable The right tympanic membrane as well as radiography of the right temporal bone and paranasal sinuses were normal On the left side there was a dry post radical mastoidectomy cavity Audiometry revealed bilateral hearing loss of 40–60 dB middle ear type (Fig 1) This was identical with the available audiograms which were done 8 3 and 1 year previously

In view of the history and lack of pathological findings an explorative tympanotomy was undertaken in the supposition that the hearing loss of the right ear could be caused by a congenital middle ear malformation

At the procedure both ankylosis of the footplate and incus fixation were found The fixation of the incus was caused by a thin piece of bone approx 2 mm long which emerged from the edge of the posterior auditory canal wall approx 1 5 mm superior to the eminentia pyramidalis and placed at right angles over the long process of the incus This bone was intimately adherent to the incus with fibrous tissue From the apex of this bone the chorda tympani nerve emerged and continued in its normal course (Fig 2)

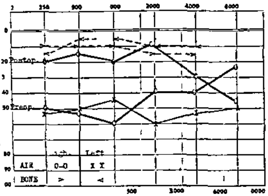


Fig 1 Preoperative and postoperative audiogram

Following removal of this bony sleeve the movements of the incus became normal During the manipulation the nerve was sectioned Afterwards a stapedectomy was performed The oval window was covered by a vein graft a 4 mm stainless steel Robinson piston was introduced and the drum replaced

The postoperative course was uneventful The audiogram performed 5 months postoperatively revealed normal hearing except for some high tone hearing loss (Fig 1)

COMMENT

Many reports have dealt with embryological etiological and clinical aspects of congenital fixation of the stapes footplate (House et al 1958 Anson et al 1960 Gundersen 1967 Steele 1969)

Clinically the known variations of chorda tympani nerve are apparently unimportant These variations are chiefly concerned with the site of the origin as related to the facial nerve *the exit from its bony canal the diameter* of the nerve and its course through the tympanic cavity (Hough 1958 Kullman et al 1971)

As opposed to this the anomaly here described contributed to the fixation of the incus Although it would appear that the main reason for the middle ear deafness was the ankylosis of the footplate it may well be that the fixation of the incus represented an additional factor

To the best of our knowledge no similar symptomatic anomaly of the chorda tympani nerve has been reported hitherto

ZUSAMMENFASSUNG

Es wird uber eine seltene Anomalie d r Chorda tympani berichtet. Man fand den hinteren Teil des Nervs der durch die Paukenhohle lauft in einer knochernen Umhullung Zum Unterschied zu den bekannten asymptomatischen Variationen verursachte diese Anomalie eine Fixation des Incus und infolg dessen beteilıgte sie sich an der vorhandenen Mittelohrschwerhorigkeit



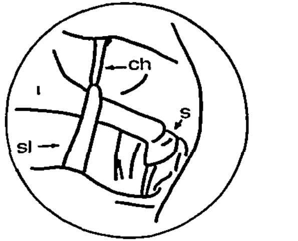


Fig 2 Photograph and schematic drawing of the middle ear demonstrating th bony sleeve" of the chorda tympani nerve *i* incus *s* stapes *ch* chorda tympani *sl* bony sleeve"

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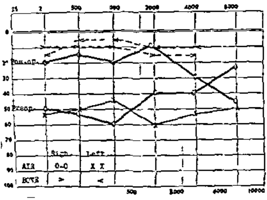


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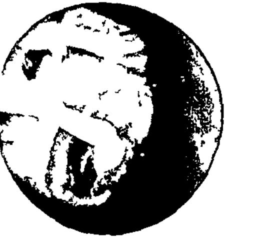
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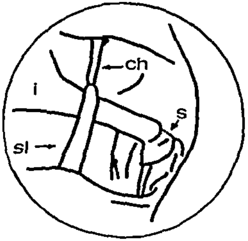


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ELECTROMYOGRAPHIC STUDIES OF FACIAL MUSCLE ACTIVITY IN SPEECH

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Abstract The function of the lip muscles during co articulation of different labial speech sounds has been studied electromyographically with concentric needle electrodes in a subject with phonetically and acoustically normal speech Polygraphic EMG re cording made it possible to analyse in detail the participation of the various muscles in the articula tory movements of the lips for the labial speech sounds The EMG pattern was characteristic of a particular speech sound in one and the same context and highly reproducible in all the experiments un dertaken Two different types of EMG activity could be distinguished 1) speech posture i e background activity which was most pronounced in M lev lab and M dep lab and appeared both before during and between the different utterances and 2) articula tory activity which was associated with the rapid movements during utterances and occurred in all the muscles examined On the basis of this latter activity it was possible to divide the labial musculature into two antagonistically active groups of muscles which appeared to receive reciprocal innervation one for rounding or closing the other for opening or spreading speech gestures Motor unit activity in a particular muscle was not constant for a given speech sound but varied with both the intensity and the duration of the activity during the preceding sound

The electromyographic study of the labial mus culature was introduced by Lysaught et al (1961) to complement previous acoustic and phonetic methods of speech analysis It was expected that this more direct means of study would lead to a clearer understanding of the relation between the neuro-muscular basis of articulatory movements and the discrete units of phonetics The results however have not really come up to expectation partly because the elec trode technique previously employed using one or two surface electrodes did not provide suffi cient resolution for a study of the pattern of neuro-motor activity in individual facial muscles (Harris et al 1962 MacNeilage 1963 From kin 1966)

The purpose of the present investigation has been to plot the function of the lip muscles in different labial articulatory movements by means of electromyography with concentric needle electrodes Particular attention has been devoted to the interaction between different muscles and groups of muscles Some of the results have been presented in preliminary re ports (Leanderson et al 1967 Ohman 1967)

This investigation was supported by grants from Stif telsen Therese och Johan Anderssons minne and Reservationsanslaget

METHODS

The EMG activity was monitored simultane ously in 6 perioral muscles (Fig 1) with fine, concentric needle electrodes (DISA 13 K 59 diam 0 30 mm) suitably amplified (Grass pre amplifier P 9) and recorded at 9 5 cm/sec on a multichannel tape recorder (P I 6200) to- gether with the acoustic signal The signals were then played back at 0 95 cm/sec and tran scribed at a paper speed of 10 mm/sec

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ELECTROMYOGRAPHIC STUDIES OF FACIAL MUSCLE ACTIVITY IN SPEECH

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(Received February 15 1971)

Abstract The function of the lip muscles during co-articulation of different labial speech sounds has been studied electromyographically with concentric needle electrodes in a subject with phonetically and acoustically normal speech Polygraphic EMG recording made it possible to analyse in detail the participation of the various muscles in the articulatory movements of the lips for the labial speech sounds The EMG pattern was characteristic of a particular speech sound in one and the same context and highly reproducible in all the experiments undertaken Two different types of EMG activity could be distinguished 1) speech posture i e background activity which was most pronounced in M lev lab and M dep lab and appeared both before during and between the different utterances and 2) articulatory activity which was associated with the rapid movements during utterances and occurred in all the muscles examined On the basis of this latter activity it was possible to divide the labial musculature into two antagonistically active groups of muscles which appeared to receive reciprocal innervation one for rounding or closing the other for opening or spreading speech gestures Motor unit activity in a particular muscle was not constant for a given speech sound but varied with both the intensity and the duration of the activity during the preceding sound

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The EMG activity was monitored simultaneously in 6 perioral muscles (Fig 1) with fine concentric needle electrodes (DISA 13 K 59 diam 0 30 mm) suitably amplified (Grass preamplifier P 9) and recorded at 9 5 cm/sec on a multichannel tape recorder (P I 6200) together with the acoustic signal The signals were then played back at 0 95 cm/sec and transcribed at a paper speed of 10 mm/sec

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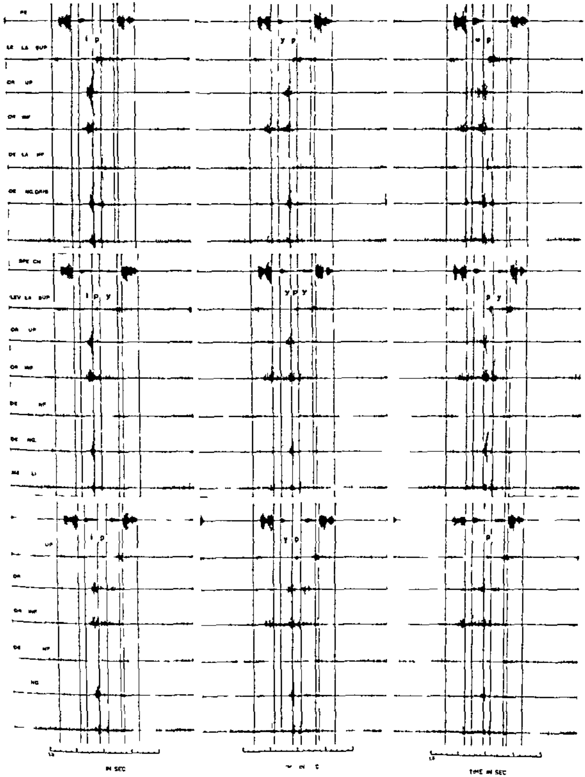


Fig 2 Speech signal and EMG activity recorded during 9 different VCV utterances embedded in the frame /sɪja dV CV dare/ The display is arranged so that the initial vowel is the same in the column while the row has the same final vowel The interv consonant is /p/

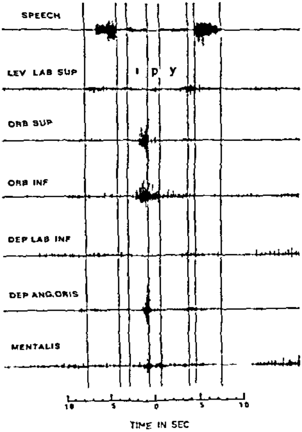


Fig 3 EMG activity during [ipy] (detail from Fig 2)

tic segment of the utterance This activity varied somewhat in intensity and duration between utterances depending upon the pre- position of the lips

The articulatory activity of the different muscles

It was possible with the aid of the segment boundaries (see Methods) to relate the EMG activity in the various muscles associated with different phonemes to the acoustic signal Analysis of the polygraphic displays of the different utterances (Fig 2) permitted the identification of the muscles used in the production of the labial phonemes investigated

For the rounding gestures of [y] and [u] EMG activity can be seen in M orb oris sup, M orb oris inf M dep ang oris and M mentalis For the spreading gesture of [i] activity appears in M lev lab and M dep lab

The production of [p] demands two opposite gestures For the closing or implosive phase a burst of activity is visible in the above mentioned rounding muscles For the opening or release phase the activity is shifted to the spreading muscles accompanied by a new activity burst in M mentalis The presence of an activity burst in M lev lab and to a smaller degree in M dep lab at the [p] release not followed by a spread vowel indicates that this phase is brought about by a specific active command and is not merely a passive result of the cessation of the closure command or the activity of the post-consonantal vowel

The latency between the onset of activity in a certain muscle and of the corresponding acoustic segment the anticipation time was found to be twice as long for a rounded vowel as for a stop consonant about 200 msec as against 100 msec

Further analysis of the EMG activity shows that the various muscles can be divided into two functionally antagonistic groups One group comprises the lip-rounding and lip-closing M orb oris sup M orb oris inf and M dep ang oris the other the lip-opening and lip-spreading M lev lab and M dep lab One of the muscles M mentalis is activated for both the implosion and explosion of [p] i e for opposite articulatory movements

Reciprocal innervation

At the onset of the bilabial stop consonant the activity in the closing muscle group increases (Fig 2) At the same time the activity in the opening group disappears or decreases espe

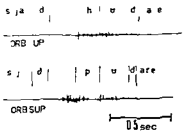


Fig 4 EMG activity in M orb oris sup during the utterances [ihu] and [ipu] resp with inhibition during [p] release

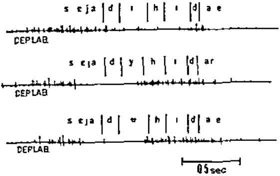


Fig 5 EMG activity in M dep lab inf during utterances with the final [ı] in different contextual positions

cially if these muscles contract tonically for the speech posture or are active for the preceding phoneme At the release of the stop the activity decreases in the former muscle group during a short interval of time while the activity in the latter group appears or increases

Fig 4 shows the activity in M orb oris sup during [ıhu] and [ıpu] resp The muscle is active throughout the [h] in preparation for the rounding of the following [u] This activity is temporarily inhibited however by the release of [p]

The importance of the context

The degree of motor unit activity in a muscle used for the production of a certain speech sound was strongly dependent on how much that muscle was activated during the preceding sound (cf M dep lab traces associated with a final [ı] in Fig 5) For the final as well as the initial [ı] in [ıhı] there is little or no activity after

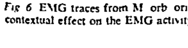


Fig 6 EMG traces from M orb oris inf showing the contextual effect on the EMG activity for the final [y]

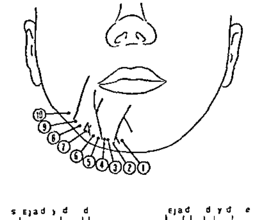


Fig 7 EMG recordings from electrodes placed in the chin muscles according to schematic drawing (the signals were rectified and smoothed—50 msec time constant—and averaged for five readings of the same utterance)

the spreading activity for [sɛja] In [yhı] and [uhı] however a burst of activity starts about 200 msec before and then declines somewhat during the [ı] This is no doubt due to the rounded lip position for the preceding vowel which requires inhibition of this muscle Another example of the importance of the context is shown in the recordings from M orb oris

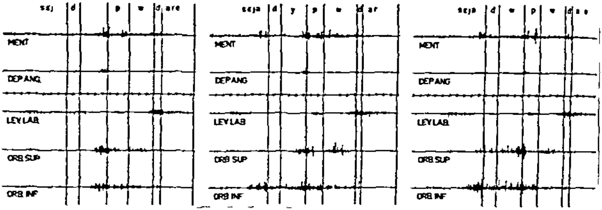


Fig 9 EMG traces with [p] in different vocalic contexts. The activity for [p] starts earlier after [i] than after [y] or [u]

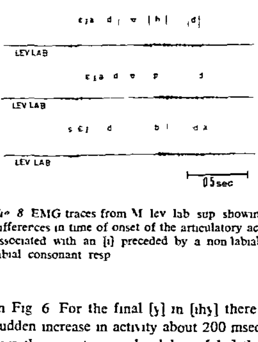


Fig 8 EMG traces from M lev lab sup showing the differences in time of onset of the articulatory activity associated with an [i] preceded by a non labial and labial consonant resp

in Fig 6 For the final [y] in [ihy] there is a sudden increase in activity about 200 msec before the acoustic signal while in [yhy] the activity is unchanged or sligthly decreased

The averaged EMG activity for the two utterances in Fig 7 obtained in an earlier study (Leanderson et al 1967) illustrates the influence of the context on the muscle activity for a phoneme in different positions In [sɛja d y d i dare] the rounded vowel segment is anticipated by a weak burst of activity at electrode positions (Ep) 5–9 associated with M dep ang oris Just before the following spread vowel [i] a more prominent activity appears at Ep 2–6 i e in M dep lab When the rounded and unrounded vowels occur in the opposite order however as in [sɛja d i d y dare] the pattern at Ep 5–9 is shifted over to the right together with the rounded vowel while the peak at Ep 2–6 earlier associated with the spread vowel has now disappeared This feature is due to the fact that in this position [i] is preceded by [sɛja] which has already caused a lip-spreading activity The last peak at Ep 2–6 more pronounced after the rounded [y] reflects the activity o[f] M dep lab during the lip-spreading gestur[e] for [dare]

During the articulation of a stop consonant the same dependence on or susceptibility to th[e] context or the muscle activity for the surroun[d]ing vowels is found (cf the EMG displays [of] [ipi] and [upu] in Fig 2) The M orb oris su[p] traces show more implosive activity for [p] aft[er] the spread vowel [i] than after the rounded [u] for which sound the muscle is already acti[ve] in an articulatory position which approach[es] that of [p] more closely

The release of the stop consonant varies ev[en] more with the context than does the implosio[n] Thus M lev lab is far more active for release if the post-consonantal vowel is spre[ad] than if it is rounded (Fig 2)

The length of time by which EMG activi[ty] preceded the acoustic segment the anticipati[on] time varied for different phonemes and for t[he] same phoneme in different positions Fig [8] compares the M lev lab pattern for [ul] [upi] and [ubi] The activity burst associat[ed]

with the final [i] is delayed in the utterance containing a labial stop consonant because of the inhibition during the [p]- or [b] implosion It starts about 200 msec before the onset of [i] preceded by a non labial consonant as against c 100 msec when the intervocalic consonant is [p] or [b] The activity for [p] e g in M dep ang. oris (Fig 9) starts earlier after preconsonantal [i] than after [y] and later still after [u] whose labial position contrasts less with that of [p]

DISCUSSION

The present investigation shows that polygraphic electromyography with fine concentric needle electrodes in the labial musculature gives a characteristic and reproducible pattern for a particular phoneme in the same context when produced by the same subject in different experiments This technique appears to be a practical means of defining the movements of the lips that occur during articulation While these observations were made in but one subject a preliminary analysis of results obtained with the same technique in 10 other subjects with normal speech reveals essentially similar EMG patterns in spite of the occurrence of individual variations (Leanderson in preparation)

Electromyography has previously been used for investigation of facial muscles during speech for instance by Harris et al 1962 MacNeilage 1963 Fromkin 1966 Most of these experiments were performed with surface electrodes Although this technique may be useful for a rough assessment of the function of an entire muscle or group of muscles the complex topographical anatomy of the labial musculature with several overlapping muscles with different functions makes it impossible to relate EMG activity to a particular muscle In contrast needle electrodes facilitate both a spatial and temporal resolution of the activity of individual muscles in close proximity to one another The use of needle electrodes would therefore appear to be essential for the detailed analysis of the function of the various labial muscles

The question may of course be raised does the needle electromyography give a representative picture of the activity of a muscle? It is now clearly established that the separate muscle fibres of a motor unit are widely dispersed in the cross section of a muscle (Edstrom & Kugelberg 1968) Thus an electrode placed more or less in the centre of a muscle gives a representative picture of motor unit density in the muscle On the other hand it is somewhat hazardous to use EMG activity as a measure of the force developed in the muscle because the linear relation between the integrated electrical activity of the muscle and its tension only applies during isometric contraction (e g, [illegible] & Lippold 1954)

The phonetic material used in previous experiments has consisted mainly of [illegible] utterances The consonant and vowel phonemes studied have been placed in VCV [illegible] CVC combinations respectively In utter[illegible] of this kind however we found it diff[illegible] distinguish between EMG activity dire[illegible] sociated with labial articulation and [illegible] activity These difficulties were [illegible] placing the phonemes to be studied [illegible] tic frame which also prevented [illegible] tion and over emphasis of the [illegible] study In this way experimental [illegible] made to bear a closer resembl[illegible] speech as regards tempo rhythm [illegible] tory effort.

Analysis of EMG activity sh[illegible] possible to distinguish between [illegible] groups of muscles M orb or[illegible] oris inf on one hand and M [illegible] lab on the other Between [illegible] were signs of reciprocal inn[illegible] in one muscle reduced th[illegible] activity tagonist (e g for the [p]-[illegible] in its an tence of reciprocal innerva[illegible] The existional organization in the [illegible] is a fun- labial musculature resem[illegible] control of the muscles [illegible] that of the [illegible]

The background acti[illegible] ture and occurred both be[illegible] of a [illegible] utterances An increase of activity [illegible]

latency before the utterance indicated that the subject prepared himself to speak by first assuming a basic speech posture Other investigators (e g Björk 1961 Fritzell, 1969) have found similar prespeech adjustments in other speech muscles In this study the background activity was most apparent in M lev lab and M dep lab It is not yet known whether these muscles have a different spectrum of motor units than the orbicularis musculature e g with a predominance of tonic motor units

Many experiments have been conducted some of them using EMG technique to ascertain whether one and the same phoneme is correlated to one and the same motor command at the peripheral neuro-muscular level. In keeping with the linguistic theories then current the Haskins group (Harris et al 1962) found the same EMG pattern for one and the same phoneme regardless of the surrounding phonemes. MacNeilage (1963) found that the muscle action patterns associated with a phoneme were independent of the context and concluded that the contextual effect was an acoustic property alone In subsequent studies (MacNeilage & Sholes 1964 Fromkin 1966) the validity of these observations have been questioned and it has been shown that there is no one-to-one relationship between phonetic units and neuro-muscular activity

We found that the degree of motor unit activity in a muscle used for the production of a certain speech sound was strongly dependent on how much that muscle was activated during the preceding sound.

Whether this dependence on context requires peripheral feedback mechanisms e g of the gamma loop type or is centrally learned (Persson et al. 1969) has been the subject of another investigation which will be described in a subsequent report.

ZUSAMMENFASSUNG

Die Funktion der Lippenmuskulatur bei der Koartikulation verschiedener labialer Sprachlaute wurde bei einer Person mit phonetisch und akustisch normaler Sprache elektromyographisch mit Hilfe konzentrischer Nadelelektroden untersucht. Die polygraphische EMG Aufzeichnung ermöglichte eine Detailanalyse der Beteiligung der verschiedenen Muskeln am artikulatorischen Bewegungsablauf der Lippen bei der Bildung labialer Sprachlaute Die EMG Kurven waren charakteristisch für einzelne Sprachlaute in ein und dem selben Kontext und in allen vorgenommenen Versuchen im hohen Masse reproduzierbar Im EMG konnten zwei Aktivitätstypen unterschieden werden. 1) die Spracheinstellung, d. h. die Hintergrundsaktivität, die am meisten im M lev lab und M dep lab ausgeprägt war und deutlich wurde vor während und zwischen den verschiedenen Lautbildungen und 2) die Artikulationsaktivität die mit den schnellen Bewegungsabläufen während der Lautbildung verbunden war und in allen geprüften Muskeln auftrat. Diese letztere Aktivität ermöglichte es die labiale Muskulatur in zwei antagonistische Muskelgruppen mit reziproker Innervation einzuteilen Die eine Gruppe zum Runden und Schliessen die andere zum Öffnen und Erweitern Die EMG Aktivität war für einen gegebenen Sprachlaut in einem einzelnen Muskel nicht konstant sondern variierte mit der Intensität und Dauer der Aktivität eines vorher gebildeten Lautes

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PROPOSALS FOR THE TNM CLASSIFICATION AND ITS EXTENDED APPLICATION FOR MALIGNANT TUMOURS OF THE HEAD AND NECK REGIONS

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Abstract A proposal is made for the TNM classification system of malignant tumours of the head and neck regions, where a definite classification has not yet been determined by UICC The regions include the maxillary sinus, ethmoid cells, frontal sinus, sphenoid sinus, nasal cavity external nose middle ear external ear parotid gland submandibular gland, trachea and orbit. The extent of the primary tumour is divided into four grades. Although the criteria for application of this grading are different from one region to another the common principle of the classification is as follows.

T1 Tumour of 2 cm or less in its greatest dimension and tumour without deep infiltration.

T2 Tumour of more than 2 cm but not more than 4 cm in its greatest dimension, or tumour with deep infiltration but confined to the primary region with the external periosteum or the capsule of the gland remaining intact.

T3 Tumour of more than 4 cm but not more than 6 cm in its greatest dimension, or tumour extending beyond the primary region through the external periosteum or the capsule of the gland

T4 Tumour of more than 6 cm in its greatest dimension, or tumour extending to main neighbouring structures such as the base of skull, cranial cavity cervical vertebrae deep cervical muscles, carotid artery and thoracic cavity

For N and M the same criteria are used as in the UICC classification

On the premise of recording by computer the classification numbers of all malignant tumours according to the TNM system are recorded as the code numbers. Wide utilization of the classification is proposed by chronological and serial recording of the findings by codes during the course of treatment and follow-up.

The classification of malignant tumours by the TNM system has been internationally established and is already in wide use In the otolaryngological field, TNM clasifications have been established for carcinoma of the buccal cavity pharynx larynx thyroid gland and esophagus but not as yet for the nose ear salivary glands and trachea Neither has attention been paid to the ophthalmological regions. The relative rarity of carcinoma in these regions except for carcinoma of the maxillary sinus may be a contributary factor for the lack of enthusiasm for establishing a standard classification but it is just because of this rarity of cases that cooperation is necessary between cancer intitutions to bring the cases together and exchange data in a certain standardized language

It is very useful to code the findings concerning malignant tumours in order to obtain more accurate records and to facilitate statistical study particularly for processing by computer

In addition the chronological follow up of the tumour case would be greatly facilitated by describing tumours according to TNM classification codes at the time of the first examination and during the course of follow up

In this paper the following suggestions are made for establishing a TNM classification in the hope that it will be widely applied for describing the extension of the tumour during the clinical course

Principle of the classification

The essential rule of the system is that it is applied to those cases not previously treated

and that the extent of the disease must be de termined by clinical examination only Clinical examination includes diagnostic radiography or various kinds of endoscopy The present classi fication for tumours of the buccal cavity pha rynx and larynx can be applied only to those cases which have been proved histologically to be carcinoma Thus histological examination is necessitated for the classification of head and neck tumours An exploratory operation is therefore undertaken in some cases the dia gnosis can be made only after removal of the tumour In such cases the operation can be or may be considered as a part of the clinical examination

The extent of the primary tumour repre sented by T is divided into four grades in most regions Although the criteria for application of this grading are different from one region to another the common principle of the clas sification according to the proposal is as fol lows

- T1 Tumour of 2 cm or less in its greatest di mension and without deep infiltration
- T2 Tumour of more than 2 cm but not more than 4 cm in its greatest dimension or tumour with deep infiltration but confined to the primary region
- T3 Tumour of more than 4 cm but not more than 6 cm in its greatest dimension or tumour extending beyond the primary re gion
- T4 Tumour of more than 6 cm in its greatest dimension or tumour extending to the main neighbouring structures such as base of skull cranial cavity cervical vertebrae deep cervical muscles carotid artery and the thoracic cavity

In addition two other categories are used if necessary

- T1S Pre invasive carcinoma so-called carci noma in situ
- T0 No evidence of primary tumour (i e ca ses which can only be diagnosed histologically)

Concerning metastasis NM classification of the malignant tumours of the head and neck regions by UICC is employed

Regional lymph nodes are represented by N To divide the N1 or N2 categories into the subgroups (*a*) and (*b*) according to UICC seems to be meaningless

N Regional lymph nodes

- N0 No palpable nodes
- N1 Movable homolateral nodes
- N2 Movable contralateral or bilateral nodes
- N3 Fixed nodes

M Distant metastases

- M0 No evidence of distant metastases
- M1 Distant metastases present

The microscopic involvement of regional lymph nodes by tumour may be indicated by ad ding to the N category the symbol + (plus) or − (minus) depending upon the presence or absence of metastasis

The classification of the stages of disease has not yet been established except for some areas covered by the UICC system We use the clas sification system suggested by Schwab for tu mours of the head and neck regions

Stage I	T1N0M0	
Stage II	T2N0M0	
Stage III	T3N0M0	T1–3N1–2M0
Stage IV	T4N0–2M0	T1–4N3M0
		T1–4N0–3M1

To extend its application we can use the TNM system for all malignant tumours with more detailed descriptions of the histology We may apply this system without any difficulty except in cases of multiple sarcoma and sys temic diseases

Furthermore we can apply the TNM system to describe changes of findings during treat ment and to record the clinical course with the corresponding date When applied we propose to call it the T'N M system in order to distin guish it from the original TNM system T 0 N 0 or M 0 means the absence of pri

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PROPOSALS FOR THE TNM CLASSIFICATION AND ITS EXTENDED APPLICATION FOR MALIGNANT TUMOURS OF THE HEAD AND NECK REGIONS

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Abstract A proposal is made for the TNM classification system of malignant tumours of the head and neck regions where a definite classification has not yet been determined by UICC The regions include the maxillary sinus ethmoid cells frontal sinus sphenoid sinus nasal cavity external nose middle ear external ear parotid gland submandibular gland trachea and orbit The extent of the primary tumour is divided into four grades Although the criteria for application of this grading are different from one region to another the common principle of the classification is as follows

T1 Tumour of 2 cm or less in its greatest dimension and tumour without deep infiltration

T2 Tumour of more than 2 cm but not more than 4 cm in its greatest dimension or tumour with deep infiltration but confined to the primary region with the external periosteum or the capsule of the gland remaining intact

T3 Tumour of more than 4 cm but not more than 6 cm in its greatest dimension or tumour extending beyond the primary region through the external periost um or the capsule of the gland

T4 Tumour of more than 6 cm in its greatest dimension or tumour extending to main neighbouring structures such as the base of skull, cranial cavity cervical vertebrae deep cervical muscles carotid artery and thoracic cavity

For N and M the same criteria are used as in the UICC classification

On the premise of recording by computer the classification numbers of all malignant tumours according to the TNM system are recorded as the code numbers Wide utilization of the classification is proposed by chronological and serial recording of the findings by codes during the course of treatment and follow-up

The classification of malignant tumours by the TNM system has been internationally established and is already in wide use In the otolaryngological field TNM clasifications have been established for carcinoma of the buccal cavity pharynx larynx, thyroid gland and esophagus but not as yet for the nose ear salivary glands and trachea Neither has attention been paid to the ophthalmological regions. The relative rarity of carcinoma in these regions except for carcinoma of the maxillary sinus may be a contributary factor for the lack of enthusiasm for establishing a standard classification but it is just because of this rarity of cases that cooperation is necessary between cancer intitutions to bring the cases together and exchange data in a certain standardized language

It is very useful to code the findings concerning malignant tumours in order to obtain more accurate records and to facilitate statistical study particularly for processing by computer *In addition the chronological follow up* of the tumour case would be greatly facilitated by describing tumours according to TNM classification codes at the time of the first examination and during the course of follow up

In this paper the following suggestions are made for establishing a T N M classification in the hope that it will be widely applied for describing the extension of the tumour during the clinical course

Principle of the classification

The essential rule of the system is that it applied to those cases not previously treated

3 Tumour infiltrating into the contralateral frontal sinus or into the surrounding tissue beyond the frontal bone
4 Tumour extending to the cranial cavity by penetration of dura mater

Sphenoid sinus

1 Tumour confined to the sphenoid sinus with no evidence of bony involvement
2 Tumour causing destruction of the sphenoid bone but not extending beyond the midline of the sphenoid sinus with the external periosteum remaining intact
3 Tumour infiltrating into the contralateral sphenoid sinus or into the surrounding tissue beyond the sphenoid bone
4 Tumour extending to the cranial cavity by penetration of the dura mater

Nasal cavity excluding the nasal vestibule

1 Tumour of 2 cm or less in its greatest dimension confined to the nasal cavity without involvement of the bone or cartilage
2 Tumour more than 2 cm in its greatest dimension or causing destruction of the bone or the cartilage constituting the nasal cavity with the external periosteum or perichondrium remaining intact
I3 Tumour extending to the contralateral nasal cavity the ipsilateral maxillary sinus or the external nose
I4 Tumour extending to the base of skull or the nasopharynx

6 *External nose including the nasal vestibule*

T1 Tumour of 2 cm or less in its greatest dimension strictly superficial and without deep infiltration into the dermis
T2 Tumour of more than 2 cm but not more than 4 cm in its greatest dimension or with infiltration of the nasal cartilage
T3 Tumour of more than 4 cm but not more than 6 cm in its greatest dimension or infiltrating the nasal bone or the maxillary bone
T4 Tumour of more than 6 cm in its greatest dimension

7 *Middle ear including the bony part of auditory canal*

T1 Tumour confined to the middle ear cavity or the bony part of auditory canal with minimal bony involvement such as disruption of the ear ossicles or the wall of each mastoid cell
T2 Tumour causing destruction of the temporal bone with the external periosteum remaining intact
T3 Tumour infiltrating into the surrounding tissue beyond the temporal bone or with facial nerve palsy
T4 Tumour extending to the cranial cavity by penetration of the dura mater

8 *External ear excluding the bony part of auditory canal*

T1 Tumour of 2 cm or less in its greatest dimension, strictly superficial and without deep infiltration into the dermis
T2 Tumour of more than 2 cm but not more than 4 cm in its greatest dimension or with infiltration of the auricular cartilage
T3 Tumour of more than 4 cm but not more than 6 cm in its greatest dimension or infiltrating the temporal bone or the parotid gland
T4 Tumour of more than 6 cm in its greatest dimension

9 *Parotid gland*

T1 Tumour of 2 cm or less in its greatest dimension and confined to the parotid gland without facial palsy
T2 Tumour of more than 2 cm but not more than 4 cm in its greatest dimension with the capsule remaining intact or with palsy of one of the facial nerve branches
T3 Tumour of more than 4 cm in its greatest dimension or infiltrating into the surrounding tissue through penetration of the capsule or with palsy of all of the facial nerve branches

T4 Tumour extending to the mandible the base of skull or the carotid artery

10 *Submandibular gland*

T1 Tumour of 2 cm or less in its greatest dimension and confined to the submandibular gland

T2 Tumour of more than 2 cm but not more than 4 cm in its greatest dimension with the capsule and mobility remaining intact

T3 Tumour of more than 4 cm in its greatest dimension or infiltrating into the surrounding tissue through penetration of the capsule with lack of mobility

T4 Tumour extending to the mandible or the carotid artery

Notes Malignant tumours of the sublingual gland are not considered because the gland is small and they are practically included in the malignant tumour of the floor of the mouth

11 *Trachea*

T1 Tumour of 2 cm or less in its greatest dimension and confined to the inside of the trachea

T2 Tumour of more than 2 cm in its greatest dimension or causing destruction of the cartilage rings of the trachea

T3 Tumour infiltrating slightly into the subglottic cavity thyroid gland or the other surrounding tissue beyond the trachea

T4 Tumour extending to the esophagus the deep cervical muscles or the carotid artery

12 *Orbit*

T1 Tumour confined to the primary site such as the palpebra the lachrymal gland or the lachrymal sac

T2 Tumour infiltrating into the orbit without involvement of the orbital bone

T3 Tumour causing destruction of the bony wall constituting the orbit and infiltrating the surrounding tissue

T4 Tumour extending to the cranial cavity by penetration of the dura mater

Consideration of the Classification

Concerning the classification of head and neck tumours by the TNM system Lederman (1965 1969) Schwab (1968) Pascher (1968) Sisson (1963 1965) Wustrow (1965 1968) and Cochi (1960) have publicized reports Since 1966 the Committee of Classification of Maxillary Cancer has discussed the classification system in Japan This time the author Sakai as one of the members of the Committee has made a further attempt to classify malignant tumours of maxillary antrum ethmoid cells frontal sinus sphenoid sinus nasal cavity external nose middle ear external ear parotid gland submandibular gland trachea and orbit In planning this proposal an attempt has been made to provide criteria which are objective readily understandable and at the same time to arrange factors common to the treatment and the prognosis

According to the principle of the classification tumours are divided into four groups T1 through T4 using their greatest dimensions of 2 4 and 6 cm as the borderlines The greatest dimension of a tumour is usually difficult to measure directly, except for those tumours which are superficial or exophytic or which are examined postoperatively on the cut surfaces However it is usually possible to estimate the approximate size of a tumour by clinical examination utilizing tomograms and contrast radiograms etc Naturally topographic specificity should be always taken into consideration along with the size of the tumour

Since the above mentioned regions are limited from the adjacent tissue by the periosteum or the capsule of the gland a distinction between T2 and T3 is made according to whether or not the tumour infiltration is beyond the periosteum or the capsule T4 classifies tumours infiltrating adjacent structures such as base of skull cranial cavity carotid artery cervical vertebrae deep cervical muscles and thoracic cavity

The greatest dimension of a tumour apparently correlates to the radio-sensitivity and the infiltration to the important adjacent organs to

the operability In other words T1 represents cases discovered in the early stage T2 locally infiltrating cases T3 advanced cases T4 inoperable advanced cases

From this standpoint we find some points of inconsistency even in the present classification system For example in malignant tumours of the oral cavity T1 is classified as a tumour measuring 2 cm or less in its greatest dimension strictly superficial or exophytic and T2 as a tumour measuring 2 cm or less in its greatest dimension with minimal infiltration in depth This kind of classification however presents a problem to us as it is not worth the practical use The T4 group in the present classification system includes some of the operable cases In the classification of malignant tumours of pharynx and larynx it is important to define in advance the site and the extent in which the tumour is confined We could point out a contradiction that if a tumour is small but spreads over two or three regions the extent of tumour infiltration becomes consequently larger than expected

In addition determination of the exact site of the tumour is sometimes difficult as the border of the site is vaguely defined These defects are shown by Sisson (1963 1965) and Wustrow (1965 1968) in the classification of maxillary cancer The cases classifed as T4 comprised 75 of all cases by Sisson whereas T1 and T2 cases were only 4% by Wustrow (1965, 1968)

In the classification of external ear cancer by Lederman (1965) and Pascher (1968) the auricle and the external auditory canal were divided into many sites though this seems to have little value These problems mentioned will be settled one by one in the near future and we hope our proposal in this paper might improve the present situation

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ZUSAMMENFASSUNG

Ein Vorschlag zur Anwendung des TNM Systems bei bosartigen Tumoren im Kopf und Hals Bereich wo die Richtlinien der UICC bisher noch fehlen wird hier geschildert In diesem Bereich sind Oberkiefer hohle Siebbeinzellen Stirnhohle Keilbeinhohle Nasenhohle außere Nase mittleres Ohr außeres Ohr Parotis submandibulare Speicheldruse Trachea und Orbita eingeschlossen Nach der Ausdehnung des primaren Tumores wird dieser in die vier Kategorien (T1–T4) eingeteilt Obwohl die einzele Klassifizierung des Tumores je nach den Regionen verschieden ist findet sich ein gemeinsames Prinzip der Klassifikation wie folg

T1 Der Tumor misst 2 cm oder weniger in seiner grossten Ausdehnung und ist ohne Tiefenwachstum

T2 Der Tumor misst mehr als 2 cm aber nicht mehr als 4 cm in seiner grossten Ausdehnung oder er hat ein Tiefenwachstum jedoch noch auf die primaren Region mit intaktem Periost bzw intakter Kapsel der Druse beschrankt

T3 Der Tumor misst mehr als 4 cm aber nicht mehr als 6 cm in seiner grossten Ausdehnung oder dehnt sich uber die entsprechende Region des ausseren Periost bzw die Kapsel der Druse aus

T4 Der Tumor misst mehr als 6 cm in seiner grossten Ausdehnung oder dehnt sich auf eine der wichtigen Nachbarregionen aus wie Schadelbasis Schadelinnenraum Halswirbel tiefe Halsmuskeln A carotis bzw Thorax

N und M Kategorie stehen die ublichen Richtlinien der UICC fur das HNO-Gebiet zur Verfugung

Fur das Stadium ist die T Kategorie massgebend N1-2 werden jedoch fur Stadium III und N3 oder M1 fur Stadium IV eingeteilt

Unter der Voraussetzung dass man die Computer technik anwendet, schlagen wir vor das TNM System auch bei allen anderen bosartigen Tumoren ausser Karzinom zusammen mit Histologie Schlusseln anzuwenden Auch konnen weitere Schlussel bei der Kontrolluntersuchung zum Zeitpunkt der Befunderhebung nach dem TNM System aufgestellt werden um den gesamten Verlauf der Erkrankung zu uberblicken Es erscheint dabei zweckmassig diese neue Kategorie das T N M System zu nennen damit es von dem originalen TNM System unterschieden werden kann

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OBSERVATION ON THE ORGAN OF CORTI WITH SCANNING ELECTRON MICROSCOPE

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Abstract With the scanning electron microscope three-dimensional observations of the organ of Corti were carried out Some of the remarkable findings are presented as follows

1) The cuticular plates of the s cond and third row outer hair cells are octagonal and on the periphery of the cuticular plates there are numerous microvilli

2) The basal body of the first and second row outer hair cells is distinct but the one of the third row outer hair cells is not quite so distinct Inner hair cells have no basal body but some of them have a bump like process at the site where the basal body is supposed to be

3) Some hairs show the nodules

4) Fibers of the laminal process of the outer pillar cells are seen reaching over the cuticular plates of the outer hair cells like climbing ivy

5) The structures of the upper and lower surfaces of the tectorial membrane were observed in detail

The most characteristic feature observed is the three-dimensional finding of the hollows arranged in V shape on the lower surface of the tectorial m m brane to which the tips of hairs of the outer hair cells fit

Since Engstrom first made electron micro scopic observations on the cochlea in 1953 there appeared many reports on the fine structures of the cochlea (Engstrom & Wer sall 1953 *a* *b* 1958 Engstrom & Sjostrand 1954 Engstrom et al 1962 Engstrom et al 1966) The electron microscopic method by Engstrom et al with serial sections of the cochlea is an excellent way to approach the fine structures of the inner ear but is not quite sufficient to get the three-dimensional profile as a whole

With the scanning electron microscope in contrast it enables us to observe the surface structures of the entire tissues in three di mensions and thus the observation conducted in this manner is more suitable to get clear views in three dimensions of a complex struc ture like labyrinth Only available observa tions with the scanning electron microscope have been done by Lim & Lane (1969 *a* *b*) and Lim (1970)

In this report we intend to present our find ings by the scanning electron microscope on the fine structures of guinea pig cochlea

MATERIAL AND METHODS

The animals used were normal guinea pigs weighing 300–350 g After decapitation the cochlea was taken out as soon as possible and under the surgical microscope the stapes and the round window membrane were re moved a small hole was then made in the apex the cochlea was fixed (2 ′ osmic acid and 2 5 % glutaraldehyde) and after fixation the graded alcoholic dehydration was carried out Then the desired portions were taken out at the stage of 75 alcohol and finally de hydrated again with anhydrous acetone and dried by warm air The dried specimens were given the double coating of carbon and gold and these were observed with the scanning electron microscope JSM 2type (Japan Elec tron Opticus Lab)



1 Surface structure of the reticular lamina One of inner hair cells (*IHC*) can be seen and three (*1 2 3*) rows of outer hair cells in a distinct and orderly array and on the cuticular surface is observed a V shaped hair pattern There can also be observed laminal processes (*LP*) of the outer pillars of tongue shaped and phalanges (*D*) of the Deiters cells of a dumbbell shape as the supporting cells in between the outer hair cells Note that in the first row of the outer hair cells (1) the basal bodies (*B*) are especially distinct × 3 000

RESULTS

Outer hair cells

It has been observed that the outer hair cells are arranged in three rows of which the cuticular plate in the first row is of goblet shape and that in the second and third rows is octagonal in shape (Figs 1 2 3)

On the cuticular plate near its outer periphery there can be seen microvilli of varying size and their number increases from the first row to the third row (Figs 1 2 3) There can be observed distinctly a basal body of the cuticular plates in the first and second rows (Figs 2 3) but often not so distinct in the third row

Inner hair cells

Hairs of the inner hair cell are arranged in 3–5 rows in an echelon formation and the height as well as the size of hairs are uniform on the outer side (on the side of Hensen s

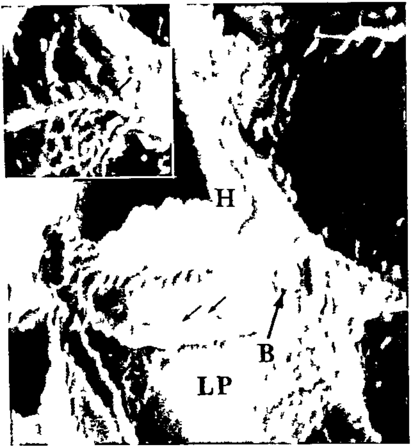


Fig 2 High magnification of the first row outer hair cell V haped hair pattern (*H*) can be een and the basal body (*B*) is distinct in semispherical shap The arrows indicate the fibers of laminal process of the outer pillar (*LP*) reaching over the cuticular plate of the outer hair cell like climbing ivy × 10 000 *Inset* same picture at greater magnification showing the portion where the fibers reach the cuticular plate × 18 000

cells) while on the inner side (on the side of Modiolus) the size and height of the hairs are variegated (Fig 4) Some of the hairs have nodal protrusion (arrow in Fig 4) There is also a small mound or bump on the cuticular plate near the outer periphery (Fig 5)

Supporting cells

The surface of the laminal processes of the outer pillar cells reveals numerous fibers making network and some of these fibers reach over the cuticular plate of the outer hair cells like climbing ivy (Fig 2) The phalanx of Deiters cells is of a dumbbell shape (Fig 1)

4 Tectorial membrane

On observing the contact of the tectorial membrane with the hairs the outermost end of the tectorial membrane is in a relatively close contact with hairs of the third row outer hair cells (Fig 6) There is no evidence to show that the tectorial membrane is in actual contact with Hensen s cells On viewing the lower surface of the tectorial membrane there can be observed hollows arranged in V shape which coincide with the V shaped hair pattern of the outer hair cells in the first second and third rows (Figs 7 8)



Fig 3 High magnification of the second row outer hair cell The cuticular plate is of an octagonal shape the periphery of which projects upward forming a bowl with hairs (*H*) in the center On the periphery of the cuticular plate there are numerous microvilli *B* seems to be a basal body which is not so distinct as that of the first row Note the nodules (*arrow*) of hairs × 10 000

COMMENT

The shape of the cuticular plate of the outer hair cell so far reported has been hexagonal (Nomura 1967) whereas that of the second and third row hair cell we have observed is clearly octagonal (Fig 3)

Engstrom et al (1966) state that each one of the outer hair cells has a basal body but the basal body of the third row is not so clearly observed The basal body of the first row is especially large and distinct (Fig 2)

Judging from the concept of stimulation theory (Engstrom et al 1962) of the basal body these findings seem to indicate that the first row outer hair cells receive the strongest stimulation

In the peripheral region of the cuticular plate there can be observed numerous micro villi of varying size the number of which in creases from the first row to the third row (Figs 1 3) It is interesting to note that the third row outer hair cells have indistinct basal body but numerous microvilli

Fig 4 High magnification of hairs of the inner hair cell *Inset* a schematic drawing of hairs of the inner hair cell at a greater magnification The photo shows *1 2 3* rows in an echelon arrangement and nodules (*arrow*) here and there on the hairs 18 000

Fig 5 A small bump (*arrow*) can be seen on the outer periphery of the cuticular plate of the inner hair cells *OHC* outer hair cells *P* headplates of the pillars *IHC* inner hair cell 3 000

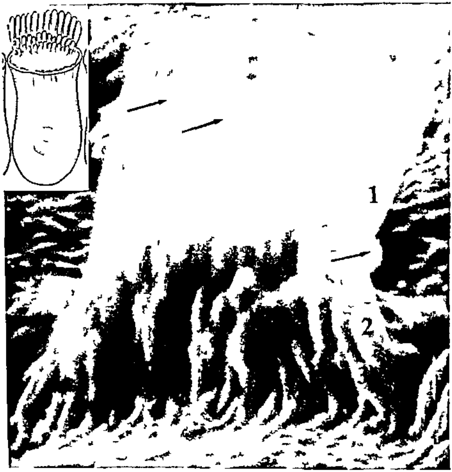


Fig 4

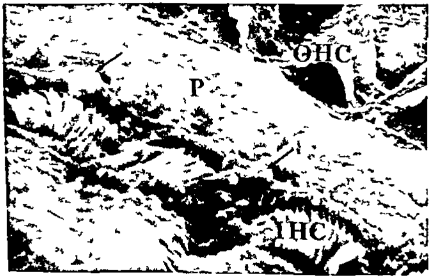


Fig 5

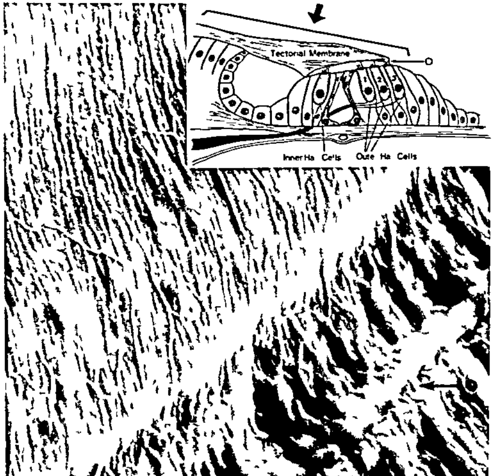

Tectonal Membrane
Inner Ha Cells
Oute Ha Cells

Fig 6

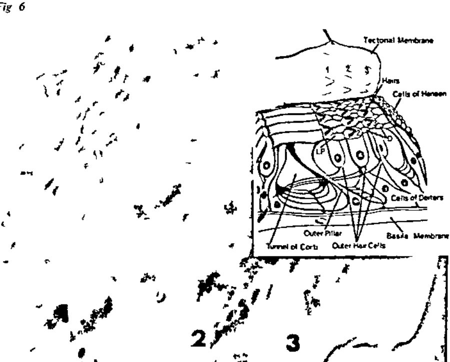

Tectonal Membrane
Hairs
Cells of Hensen
Cells of Deiters
Outer Pillar
Basila Membrane
Tunnel of Corti
Outer Hair Cells
2
3

Fig 7



Fig 8 Magnification of the blocked portion □ of the picture in Fig 7 Here can be seen clearly each of the hollows that receive the tip of one the hairs of outer hair cells × 10 000

The hairs of the inner hair cell are arranged in stair formation in 3–5 rows and these hairs have nodules here and there (Fig 4) To our knowledge there has been no precise description of these nodules Such nodule can also be observed in the longest hairs of outer hair cells The cause of such nodules may be interpreted as due to a mechanical factor i e the rubbing of hairs caused by hair vibration on receiving sound as blisters are formed on the hand

Kimura (1966) reports that he has observed the basal body even in the inner hair cells but in our observations we could not recognize

Fig 6 The upper surface of the tectorial membrane as observed from the direction shown in the schematic drawing Numerous fibers radiate and over them somewhat larger fibers form a network ○ indicate the portion where the hairs of third row outer hair cells contact with the outermost margin of tectorial membrane as shown in schematic drawing 1 000

Fig 7 View of the lower surface of the tectorial membrane Coinciding with the V shaped hair pattern of the outer hair cells of the 1st 2nd and 3rd rows there can be observed hollows arran ed in V shape on the lower surface of tectorial membrane *1 2 3* in the photo correspond to 1 2 3 on the lower surface of tectorial membrane in the schematic drawing × 3 000

any basal body in the inner hair cells but a bump like process was found at the site where the basal body is supposed to be (Fig 5)

On the surface of the laminal process of outer pillar cells there are many fibers forming networks and some of these fibers extend up to the cuticular plate of the outer hair cells like climbing ivy (Fig 2) Such a structural arrangement seems to give a firm support to the outer hair cells

The phalanx of Deiters cells is of a dumb bell shape and not the knuckle shape as has been said to be (Engstrom et al 1966)

The most interesting finding of the tectorial membrane is its contact manner with hairs Recently in the electron microscopic observations of sections Kimura (1966) has observed that there are many hollows on the lower surface of the tectorial membrane and these hollows are in close contact with the tips of hairs In our observations using scanning electron microscope we have successfully observed these hollows on the lower surface of the tectorial membrane distinctly in three dimensions (Figs 7 8)

There the hollows could be recognized arranged in V shape at the lower surface of the tectorial membrane corresponding to tips of the hairs in all the first second and third row outer hair cells However we have been unable to demonstrate such hollows corresponding to the inner hair cells For a definitive answer on this point further studies are required Such a three dimensional observation on these hollows has not yet been reported

ZUSAMMENFASSUNG

Die Oberflachenstruktur des Cortischen Organs von gesunden Meerschweinchen wurde mit einem Rasterelektronenmikroskop beobachtet Besonders ging es dabei um die ausseren Haarzellen die inneren Haarzellen und die Tektorialmembran Das aufschlussreichste Ergebnis der stereoskopischen Untersuchung war dass die Haarspitzen der ausseren Haarzellen in V formig angeordnete Locher der Unterseite der Tektorialmembran eindringen aber nicht mit der Tektorialmembran verwachsen sind

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OLIVO COCHLEAR BUNDLE IN THE GUINEA PIG COCHLEA AFTER CENTRAL TRANSSECTION OF THE CROSSED BUNDLE

Electron Microscopic Study on Origin and Distribution of Unmyelinated Efferent Fibers by Axonal Degeneration

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Abstract In order to clarify origin and distribution of unmyelinated efferent fibers in the guinea pig cochlea the olivo-cochlear bundle (OCB) was electron microscopically traced from the internal auditory meatus to the organ of Corti at various intervals after central transsection of the crossed bundle In other animals the organ of Corti was destroyed in order to induce retrograde degeneration of the cochlear nerve fibers The resultant Wallerian degeneration occurred in a majority of myelinated and unmyelinated fibers in the fascicles of OCB after nerve section whereas the fascicles survived after destruction of the organ of Corti These findings indicate that both the crossed and uncrossed OCBs consist of myelinated and unmyelinated fibers Both the myelinated and unmyelinated fibers of the crossed OCB originate in the brain stem Both the crossed and uncrossed fibers are intermingled in the fascicles distal to the internal auditory meatus It is probable that the unmyelinated efferents would be different from the myelinated efferents in the functional role in hearing

The olivo cochlear bundle (OCB) efferent fibers to the cochlea (including the reticulo-cochlear bundle in this paper) has been studied by numerous investigators and has been regarded as being composed only of myelinated nerve fibers On the basis of electron microscopic observations on OCB along the course from the internal auditory meatus to the organ of Corti of the normal guinea pig cochlea we have previously reported that OCB is a mixture of myelinated and unmyelinated fibers (Terayama et al 1969) The presence of unmyelinated efferent fibers in OCB seems to be important for the following reasons 1) The myelinated and unmyelinated fibers may be different not only morphologically but also functionally 2) The ratio of the numbers of myelinated and unmyelinated *fibers in OCB is approximately 3 2 (Terayama et al* 1969) A single unmyelinated fiber contains several axons while a myelinated fiber usually contains only one If one takes into consideration that each unmyelinated fiber divides into naked axons after losing the Schwann cell sheath in the organ of Corti functional significance of the unmyelinated fibers cannot be neglected 3) The characteristic features of the efferent fibers in the organ of Corti are varicose enlargements of the preterminal fibers and accumulations of both agranulated and granulated vesicles in the enlargements (Spoendlin 1966 Smith 1967) These characteristics were also found in axons of the unmyelinated efferent fibers of OCB (Terayama et al 1969)

In our previous study identification of OCB was not conclusive because it was made mainly on the basis of the characteristic course of OCB from the internal auditory meatus to the organ of Corti According to Rasmussen

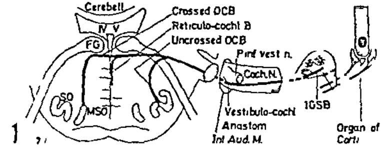


Fig 1 Schematic drawing of the course of the efferent fibers from the brain stem to the cochlea The crossed olivo-cochlear bundle (*OCB*) was cut at the floor of the fourth ventricle (*II I*) *FG* genu of the facial nerve *SO* superior olive *MSO* medial superior olive *IGSB* intraganglionic spiral bundle in the Rosenthal's canal

(1942) and Rossi & Cortesina (1963) the efferent fibers to the cochlea are composed of one large crossed and two small uncrossed bundles originating from different parts of the brain stem (Fig 1) In the present study the crossed bundle of OCB in the guinea pig was cut at the floor of the fourth ventricle in order to clarify origin and distribution of the unmyelinated fibers by means of Wallerian degeneration In addition retrograde degeneration of the afferent fibers following destruction of the organ of Corti was examined to differentiate the surviving efferent fibers in the cochlea

MATERIAL AND METHODS

Guinea pigs weighing 200–400 g were used The animals were anesthetized with sodium pentobarbital (i p 25 mg/kg) The crossed fibers of OCB were sectioned in the same way as done by Kimura & Wersall (1962) The skin of the occipital area was incised the dorsal part of the occipital bone was removed and the dura was cut While the cerebellum was lifted up under an operation microscope the fourth ventricle was opened and the cerebrospinal fluid was sucked out An incision was then made at the midline of the floor of the fourth ventricle between the facial genu The bony defect was covered by a piece of gel foam and the incised skin was sutured The operated animals were sacrificed at postoperative intervals of 1 2 3 4 5 6 8 10 13 19 37 45 and 60 days One to four animals were used for each interval and altogether 23 animals were sacrificed

In addition another 6 animals were used to examine degeneration of the afferent fibers in the cochlea A mixture containing 0 5 g of streptomycin sulfate (SM) and dihydrostreptomycin sulfate (DHSM) respectively was diluted in 10 ml of physiologic saline The perilymphatic space of the cochlea was perfused with the solution of SM and DHSM or with only the saline through the round window after opening the bulla and perforation of the stapes under general anesthesia They were sacrificed 7 12 20 35 and 64 days after the perfusion

All the animals were sacrificed by perfusing 2 5% glutaraldehyde in cacodylate buffer through the aorta under general anesthesia and artificial respiration After perfusion the brain and cochleas were removed The brain was then immersed in 10% formalin for light microscopy Serial sections of the brain embedded in paraffin were stained with Luxol Fast Blue and cresyl violet (Kluver & Barrera 1953) The bony capsule of the cochlea was widely opened and the modiolus was transversely cut into several pieces in order to facilitate diffusion of the fixative The cochlear nerve trunk was gently pushed out from the internal auditory meatus after separation from both the facial nerve and the superior division of the vestibular nerve The tissue pieces were further postfixed in 1% osmium tetroxide in phosphate buffer The techniques were previously described in detail (Terayama et al 1968 *a* 1968 *b*) The tissues were then dehydrated embedded in Epon and sectioned with an LKB Ultrotome by a diamond knife Ultrathin sections were mounted on slit meshes covered by Formvar membrane and



Fig. 2. The wound of section (*arrow*) of the brain stem at the level of the facial genu (*FG*) was filled by scar tissue and blood cells. Klüver and Barrera's stain. 19 days after nerve section. ca. × 30.

stained with uranyl acetate and lead citrate. The sections were examined by a Hitachi HS-7D or HS-11 electron microscope with magnifications of 60 to 10 000. Thicker sections were light microscopically examined after toluidine blue staining (Trump et al. 1961).

FINDINGS

The extent and depth of the incision at the floor of the fourth ventricle was light microscopically evaluated at the level of the facial genu. In most animals the crossed bundle was completely cut, though in some cases partially. The operated wound was filled with blood cells for a few days postoperatively, and then it was gradually replaced by glia cells. Nerve cells in the close vicinity of the wound degenerated or disappeared (Fig. 2).

As previously described (Terayama et al. 1969), OCB consists of 2 to 6 fascicles of nerve fibers, and it takes a peculiar course so that it is readily distinguishable from the cochlear nerve fibers in the internal auditory meatus, Rosenthal's canal and osseous spiral lamina. In these portions degeneration of nerve fibers, both myelinated and unmyelinated, were observed in all the fascicles after section of the crossed bundle of OCB. Degeneration and disappearance of nerve fibers and endings in the organ of Corti were also recognized. Degenerative changes in the organ of Corti are not mentioned in this paper since they have been already described in detail (Iurato 1962, Kimura & Wersäll 1962, Spoendlin & Gacek 1963, Smith & Rasmussen 1963).

The degenerative process of individual fibers of OCB after nerve section was various in appearance in different fibers. The degenerative signs were first found about 20 hours after nerve section in both the myelinated and unmyelinated fibers, and they be-



Fig. 3. Degenerated fibers of OCB in the internal auditory meatus 2 days after nerve section. Neurofilaments and tubules in the axons of myelinated and unmyelinated fibers disappeared (*E*) or decreased in number (*F*). Disruption of myelin lamellae (*arrows*). *V*: granulated vesicles. × 12 000.

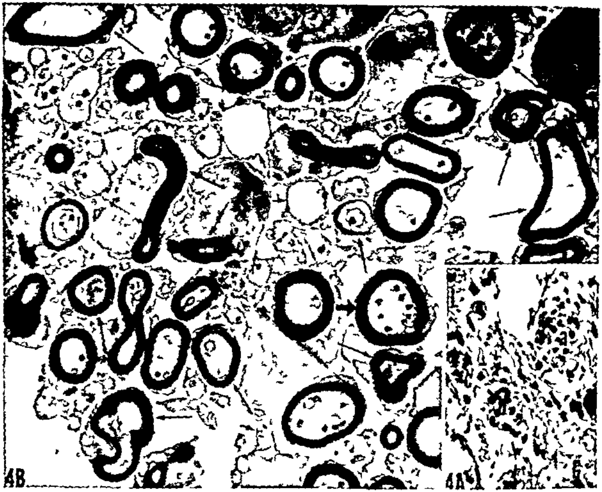


Fig 4 Intraganglionic spiral bundle Four days after nerve section (A) Light micrograph of Rosenthal s canal shows three fascicles of OCB (*a b c*) *L* osseous spiral lamina *G* spiral ganglion cells ×370 (B) Electron micrograph of fascicle *a* of Fig 4 A demonstrates many degenerating myelinated (*long arrows*) and unmyelinated (short arrows) fibers. Accumulation of mitochondria in an axon (*thick arrow*) ×5 000

came more distinct 48 hours postoperatively (Fig 3)

The earliest signs of degeneration in the myelinated fibers were the appearance of electron-dense particles of debris (Fig 5) the decrease of neurofilaments and tubules (Figs 3 4 5) and the accumulation of mitochondria in axons (Fig 4) Then 2 to 4 days postoperatively the axoplasm appeared empty due to disappearance of its content (Figs 3 4 5) In another kind of degenerative change axons were found to be filled with homogeneous material (Figs 4 5 7 8) Mitochondria in the axoplasm were swollen (Figs 3 4) In some cases moth-eaten like disruption was found in myelin lamellae (Fig 3) Five to eight days after operation the myelin lamellae began to loosen (Figs 6 8) The axon in the center of the myelin ring gradually disappeared Fragmentation of the myelin lamellae was enclosed in the enlarged Schwann cells (Figs 6 8) About 2 weeks after nerve section remnants of the myelin lamellae contained in the Schwann cells disappeared and consequently empty spaces remained inside the cells (Fig 6) The Schwann cells decreased in size and finally disappeared Thus there remained only a canal of the basement membrane which had enclosed the fiber (Figs 5 6 7 9) Later the basement membrane also disappeared

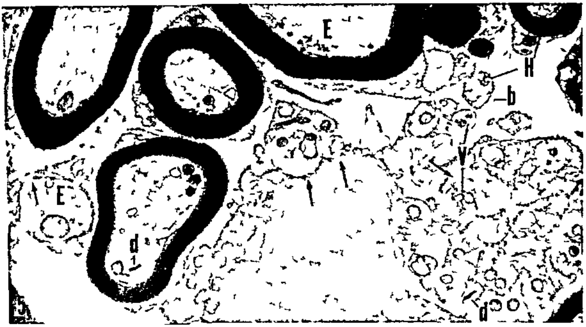


Fig. 5. Higher magnification of degenerated nerve fibers in fascicle *b* of Fig. 4 A presents empty (*E*) or homogeneous (*H*) appearance of the axoplasm of the myelinated and unmyelinated fibers. Discontinuity of the axolemma of disappearing unmyelinated fibers (*arrows*). *b*, remnants of the basement membrane; *d*, debris in the axoplasm or debris of the axon; *v*, granulated and agranulated vesicles. Note a variety of stages of degeneration in individual axons. × 12 000.

Degenerative processes of the unmyelinated fibers of OCB were essentially the same as described for the myelinated fibers. In the early stage of degeneration (Figs. 3, 4, 5, 10), accumulation of mitochondria and appearance of debris particles in axoplasm were followed by swelling of mitochondria and by emptiness of axoplasm. The axoplasm was sometimes filled with homogeneous material. In the degenerated fibers, Schwann cells were reduced in size and the axons which had been enclosed by the Schwann sheath appeared to be released. The axolemma was broken and the axons disappeared (Fig. 5). 19 to 60 days after nerve section (Figs. 6, 7, 9) the Schwann cells disappeared and only the basement membrane were recognized, which also finally disappeared. During the degeneration, phagocytes were rarely found among the degenerated fibers.

Granulated vesicles in the unmyelinated fibers of OCB, unlike other organelles in the axoplasm, remained relatively unchanged. Even 19 days after nerve section, they were found in the degenerated axons (Figs. 7, 10). In the fascicles which contained degenerated fibers, many agranulated and granulated vesicles were accumulated in the unmyelinated axons (Figs. 3, 5, 7, 9) and granulated vesicles were scattered in the myelinated axons (Fig. 8). These vesicles have been regarded as characteristic of the fibers of OCB (Terayama et al., 1969). Sixty days postoperatively, regenerating myelinated fibers were found, though rarely, in the fascicles of OCB (Fig. 9).

As shown in Fig. 10, degenerated unmyelinated fibers were occasionally found just proximal to the habenula perforata among the fibers running into the organ of Corti. However, their further peripheral course could not be traced, because in the organ of Corti all axon were naked after passing through the habenula perforata.

While degenerative changes occurred in a

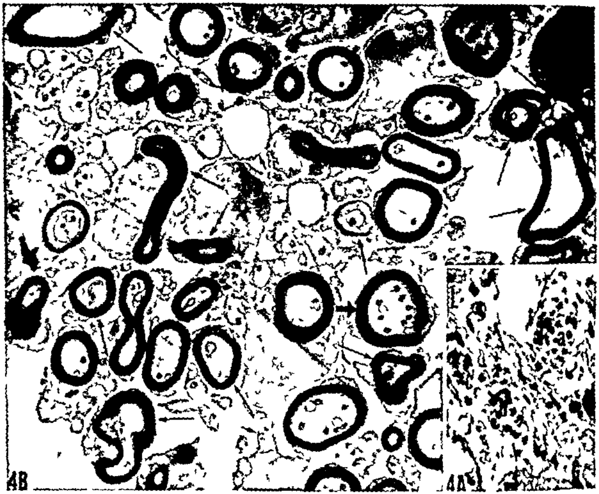


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Fig 7 Intraganglionic spiral bundle in the same cochlea as in Fig 6 Fiber population is sparse Note lack of the axons and shrinkage of Schwann cells (*S*) *b* basement membrane Granulated vesicles (*v*) still persist in the degenerated unmyelinated axon Two fibers (*arrows*) remain intact × 15 000



Fig 8 OCB in the internal auditory meatus of the same cochlea as in Figs 4 and 5 Fragmentation of myelin lamellae (*My*) and degeneration of axons (*arrows*) are more advanced centrally than peripherally Granulated vesicle (*v*) in a myelinated axon × 15 000

the fascicles of OCB did not show any de generated fibers In addition the fascicles were peculiar in the composition of myelinated and unmyelinated fibers The organ of Corti had been replaced by fibrocytes among which a small number of macrophages and of mye linated and unmyelinated nerve fibers were found The presence of such nerve fibers is of interest and it will be dealt with in detail in a separate paper

As previously reported (Terayama et al 1968) postganglionic sympathetic fibers are distributed in the guinea pig cochlea These fibers are unmyelinated and like the unmye linated efferent fibers in OCB contain ve sicles The sympathetic fibers persisted in both experiments of the present study

DISCUSSION

In order to differentiate the efferent fibers from the other kinds of nerve fibers in the cochlea there have been three methods so far available 1) Histochemical method for acetylcholinesterase (Schuknecht et al 1959 Hilding & Wersall 1962 Gacek et al 1965 Kaneko & Daly 1968 Ross 1969) or alkaline phosphatase (Hiraide 1970) 2) Survival of ef ferent fibers following retrograde degenera tion of afferent fibers due to either destruc tion of the organ of Corti (Rossi & Cortesina 1967 Kellerhals et al 1967) or selective sec tion of the cochlear nerve (Spoendlin & Ga cek 1963) 3) Wallerian degeneration of th



Fig. 9. Intraganglionic spiral bundle 60 days after nerve section. (A) Light micrograph illustrates spaces of fascicles of OCB (*a, b, c, d*). The osseous spiral lamina is at left. × 800. (B) Electron microscopic view of fascicle *a* in Fig. 9 A showing a marked decrease in the number of nerve fibers. *C*: cochlear nerve fibers. *S*: remnants of Schwann cell. *b*: basement membrane. *R*: a regenerating myelinated fiber containing myelin debris. Undegenerated fibers (*arrows*). × 4 600.

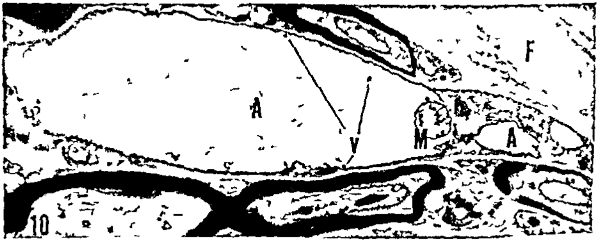
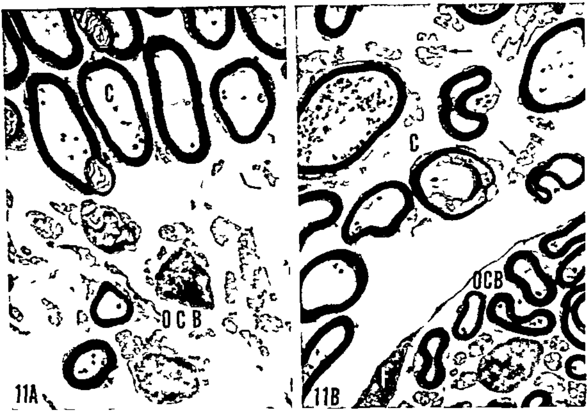


Fig. 10. A degenerated unmyelinated fiber found just proximal to the habenula perforata. Three days after nerve section. Radial section of the second turn. *A*: degenerated axons. *M*: mitochondria. *v*: vesicles. *F*: filamentous layer of the basilar membrane. × 8 000.



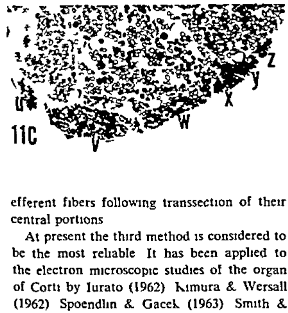


Fig 11 Comparison of OCB after central nerve section (11 A) and after destruction of the organ of Corti (11 B) at the same magnifications (× 4 000) (A) Fascicle *c* in Fig. 6 A Cochlear nerve fiber (C) remain intact with some artifacts, whereas only a few fibers of OCB persist (B) Fascicle *z* in Fig 11 C 35 days after perilymphatic perfusion with SM DHSM Cochlear nerve fibers are decreased in number whereas fibers in OCB remain intact Remnants of Schwann cells of degenerated fibers (*arrows*) The OCB is apparent *F* fibrocyte (C) Light micrograph of fascicles of OCB (*u v w x y*) in Fig 11 B × 260

efferent fibers following transsection of their central portions

At present the third method is considered to be the most reliable It has been applied to the electron microscopic studies of the organ of Corti by Iurato (1962) Kimura & Wersall (1962) Spoendlin & Gacek (1963) Smith & Rasmussen (1963) and Spoendlin (1969) These investigators could establish accurate morphology and distribution of the efferent endings and preterminal fibers in the organ of Corti in various species Their observations however were confined only to the organ of Corti except for the brief description by Kimura & Wersall on degeneration of myelinated fibers in the intraganglionic spiral bundle

The unmyelinated fibers in OCB have been previously observed but have been rather ignored Kellerhals et al (1967) found many unmyelinated fibers which were regarded as adrenergic fibers in the intraganglionic spiral bundle in the guinea pig cochlea Uchizono et al. (1967) demonstrated the presence of acetylcholinesterase positive unmyelinated fibers

in the human osseous spiral lamina but they did not mention the nature of the fibers Rasmussen (1970) observed electron microscopically numerous unmyelinated fibers in the isolated vestibulo-cochlear anastomosis of the cat

Recently fine structural changes of degeneration and regeneration of nerve fibers have been studied in various nerves of different species by many investigators (Terry & Harkin 1957 Webster 1962 Lee 1963 Nathaniel & Pease 1963 1963 Lampert & Cressman 1966 Haftek & Thomas 1968 Kruger & Maxwell 1969) In the present study a majority of myelinated and unmyelinated fibers of the fascicles of OCB were found to degenerate in the cochleas of the animals whose crossed bundle of OCB had been sectioned in the brain stem whereas these fascicles remained undegenerated in the cochleas of the animals whose organ of Corti had been destroyed Degenerative changes of both the efferent and afferent fibers in the cochlea are much the same as those of other nerves Our results confirmed that the OCB is composed of both myelinated and unmyelinated fibers

The characteristic features of the efferent fibers outside the organ of Corti have been previously described (Terayama et al 1969) 1) The myelinated efferent fibers are smaller in diameter and have more numerous Schwann cells than the cochlear nerve fibers 2) The axons of the myelinated efferent fibers contain a few granulated vesicles 3) The unmyelinated efferent fibers show axonal varicosities and contain clusters of both agranulated and granulated vesicles These characteristics were reconfirmed in this study for degenerating or surviving fibers in the fascicles of OCB

In the organ of Corti the previous denervation studies have established that on the hair cells larger nerve endings containing numerous vesicles are efferent in nature and smaller endings containing small number of vesicles are afferent (Iurato 1962 Kimura & Wersall 1962 Spoendlin & Gacek 1963 Smith & Rasmussen 1963) In addition varicose axonal enlargements with clusters of vesicles have been regarded as characteristic of efferent preterminal fibers (Spoendlin 1966 Smith 1967) The present study revealed two kinds of efferent nerve fibers outside the organ of Corti One of them is unmyelinated the axons of which resemble the efferent preterminal fibers in that they show varicose enlargements with clusters of vesicles The other is myelinated containing a few granulated vesicles The relationship of the efferent endings and preterminal fibers inside the organ of Corti to the myelinated and unmyelinated efferent fibers outside the organ still remains unknown The present study however evidenced that unmyelinated efferent fibers enter the organ of Corti because unmyelinated vesicle-containing fibers were found to degenerate just proximal to the habenula perforata after section of the crossed fibers of OCB

According to Rasmussen (1960) approximately four fifths of the fiber population of OCB originate as crossed bundle from the contralateral accessory olivary nucleus and the remaining one fifth originates as uncrossed bundle from the homolateral S shaped olivary segment The reticulo cochlear bundle arising from the homolateral reticular formation joins the uncrossed bundle (Rossi & Cortesina 1963) In each fascicles of OCB in the internal auditory meatus and Rosenthal s canal as indicated by our findings undegenerated fibers were scattered among degenerated fibers Since only the crossed bundle had been previously cut degenerated fibers in the fascicles could be regarded to belong to the crossed bundle and undegenerated fibers in the fascicles to the uncrossed bundle Furthermore both groups of the degenerated and undegenerated fibers were observed to be composed of myelinated and unmyelinated fibers From the above findings the following conclusions can be drawn The unmyelinated efferent fibers of the crossed bundle originate from the medulla oblongata Both the crossed and uncrossed bundles are composed of mye

linated and unmyelinated fibers The crossed and uncrossed fibers that arise from different origins in the brain stem are evenly contained in each fascicles and are intermingled together in the peripheral part

Making a comparison between extent of the sections in the brain stem and population of the degenerated unmyelinated fibers in the cochlea the traverse of the unmyelinated efferent fibers at the floor of the fourth ventricle seems to locate more ventrally than that of the myelinated efferent fibers It is thus possible that the myelinated and unmyelinated efferent fibers may arise respectively from different parts of the brain stem

Concerning the function of OCB electrophysiological studies have been made by numerous authors from Galambos (1956) to Pfalz (1969) It has been demonstrated that stimulation of OCB induces augmentation of cochlear microphonics suppression of cochlear action potentials (Galambos 1956) and inversion of summating potentials (Fex 1967) Thus OCB is considered to be inhibitory in nature On the other hand it is well known that physiological properties are different between myelinated and unmyelinated nerve fibers (Rushton 1951 Woodbury 1965) 1) The threshold to electric stimulation is lower in myelinated fibers than in unmyelinated 2) The impulses of myelinated fibers are conducted saltatorially whereas those of unmyelinated fibers are conducted continuously 3) Conduction velocity of myelinated fibers is proportional to the fiber diameter while that of unmyelinated fibers is proportional to the square root of the fiber diameter Thus conduction velocity in myelinated fibers is considerably faster than that in unmyelinated fibers 4) The latent period and duration of excitement in myelinated fibers are shorter than those of unmyelinated fibers

If these differences exist in the efferent fibers in the cochlea as well different functional roles would be played in the physiology of hearing by the myelinated and unmyelinated efferent fibers However no information has been available as to the functional significance of the unmyelinated efferent fibers in the cochlea

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ZUSAMMENFASSUNG

Um den Ursprung und die Verteilung der marklosen efferenten Fasern in der Meerschweinchenschnecke zu erklaren wurde da olivocochleare Bundel (OCB) vom inneren Gehorgang bi zum Cortischen Organ nach verschiedenen Zeitabstanden nach zentraler Durchtrennung des kontralateralen OCB elektronen mikroskopisch untersucht Bei anderen Tieren wurde das Cortische Organ zerstort um die cochlearen Nervenfasern retrograd degenerieren zu lassen Waller Degeneration fand in der Mehrzahl der markhaltigen sowie marklosen Fasern in der OCB Fasciculi nach Durchtrennung statt wahrend die Fasciculi nach Zerstorung des Cortischen Organs uberlebten Diese Befunde zeigen dass beide das kontra und das homolaterale Bundel des OCBs aus markhaltigen sowie marklosen Fasern bestehen Die Ursprunge von bei den den markhaltigen und den marklosen Fasern des kontralateralen OCBs liegen im Hirnstamm Die kontralateralen und homolateralen Fasern werden in der Fasciculi distalwarts vom inneren Gehorgang mit einander gemischt Es ist moglich dass die marklosen Efferente b im Horen funktionell anders als die markhaltigen Efferente sind

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THE ELASTIC PROPERTIES OF THE TYMPANIC MEMBRANE SYSTEM

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Abstract A method has been worked out for recording the volume displacement of the drum towards the closed middle ear as a function of the pressure change in the ear canal From these recordings we can calculate the elastic properties of the tympanic membrane system Studies of this kind have not been published previously In 101 cases with normal ears we found a varying degree of mobility of the drum The mobility increases in cases with a slightly reduced tubal function as compared with cases with a perfect tubal function This finding is explained by an increased pressure load on the tympanic membrane system when the pressure regulating capacity of the tube is reduced The present method seems to facilitate analysis of pathological processes in the middle ear

It is an everyday clinical experience that repeated pathological conditions like otitis and otosalpingitis often cause lesions to the elastic elements of the tympanic membrane The drum becomes flaccid and this often results in impaired hearing of the conductive type The aim of this report has therefore been to study the possibilities for a quantitative determination of the elastic properties of the tympanic membrane system on normal ears Such a study is a prerequisite for analysis of pathological ears

The tympanic membrane system is defined as the tympanic membrane with the ossicles ligaments and the middle ear muscles The middle ear system is defined as the tympanic membrane system connected to the closed middle ear cavity In order to determine the elastic properties of the tympanic membrane system—as its volume pressure relationship—it is necessary for the subject to have a perfect tubal function and thereby the ability to propagate quantitatively pressures from the rhinopharynx to the middle ear by deglutitions This is accomplished only by about 70 of subjects with normal ears and is furthermore both complicated and time-consuming Therefore by changing the pressure in the ear canal we have used the volume pressure relationship of the middle ear system instead Under certain conditions explained later this relationship differs only slightly from the volume pressure relationship of the tympanic membrane system

We have devised a method for determination of the elastic properties of the middle ear system applicable to all subjects irrespectively of the tubal function A brief account of this method is given below and the volume pressure relationships of the tympanic and middle ear systems are compared Finally the results of a study of the elastic properties of the middle ear system on 101 cases with normal ears are given and discussed

METHOD

The method published earlie by Ingelstedt et al (1967) has now been developed and permits

This work is a part report in a series of papers on the mechanics of the middle ear (Ingelstedt Ivarsson & Jonson 1967 Elner Ingelstedt & Ivarsson 1971)

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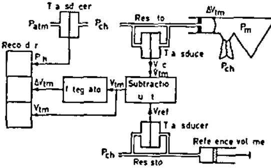


Fig 1 Block diagram of the apparatus for recording of the drum movement velocity $\dot{V}_{tm}$ and its volume displacement ΔV_{tm} at changing ambient pressure P_{ch} and middle ear pressure P_m

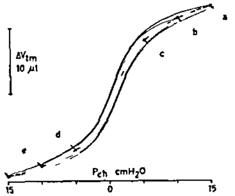


Fig 2 —— 3 recordings of the volume pressure curve of the middle ear system $\Delta V_{tm}=f(\Delta P_{ch})$ under different dynamic pressure conditions c d b-e and a f the volume pressure curve of the middle ear system under static pressure conditions

simultaneous recordings in all subjects of the movement velocity and the volume displacement of the drum not only at changes of the middle ear pressure but of the ambient pressure as well (Block diagram Fig 1) The method has been described in detail by Elner et al (1971*a*)

Volume pressure relationship of the tympanic membrane and middle ear system

The volume pressure relationship of the tympanic membrane system is defined as the volume deviation of the drum V_{tm} in relation to its neutral position as a function of pressure differences across the drum P_{tm} $V_{tm}=f(P_{tm})$ (Ingelstedt Ivarsson & Jonson 1967) The volume pressure relationship of the middle ear system is defined as the volume deviation of the drum in relation to its neutral position as a function of pressure changes in the ear canal P_c $V_{tm}=f(P\ )$ By recording volume pressure loops of the middle ear system under dynamic pressure conditions i e during changing ambient pressure $\Delta V_{tm}=f(\Delta P_{ch})$ at different pressure ranges with the tube closed and by connecting the points *a–f* (Fig 2) where the pressure is constant for 1-3 secs a volume pressure curve can be constructed This coincides with the corresponding curve recorded under static pressure conditions as shown in Table I Thus a volume pressure curve of the middle ear system for static pressure conditions can be obtained in all subjects irrespectively of their tubal capacity

If the neutral position of the tympanic membrane on this curve is known the volume deviation of the drum at different ear canal pressures can be calculated The neutral position can be determined by recording the movement velocity of the drum V_{tm} during changing ambient pressure The ambient pressure at the maximum movement velocity of the drum when moving inwards $Pch_{r_{max}}$ deviates slightly from the neutral position In cases with a perfect tubal function this deviation can be corrected individually and in other cases it is corrected by a mean value deviation of 1 cm H_2O A detailed description of this procedure is given by Elner et al (1971*c*) in a report about indirect determination of the middle ear pressure

Control experiment

In 5 subjects who could equilibrate the middle ear pressure completely the volume pressure relationship of the middle ear system obtained from the dynamic loops according to the method above was compared to the corresponding volume pressure relationship recorded in these 5 subjects under static pressure conditions (Table I) There was good agreement between these values

Table I *A comparison of volume pressure relationships $V_{tm}=f(P\ )$ on 5 cases with two different procedures of recording directly recorded r and indirectly calculated c*

Subjects	r = recorded c = calculated	P cm H O −15	−10	−5	+5	+10	+15
510917 M N	V_{tm} r	+14 8	+12 0	+7.2	−5 0	− 7 6	− 9 4
	μl c	+14 6	+12 0	+7 2	−5 1	− 8 2	− 9 9
460327 K J	V_{tm} r	+11 2	+ 9 5	+6 0	−5 0	− 8 0	− 9 6
	μl c	+11 4	+10 0	+6 7	−4 6	− 7 6	− 9.2
470206 K J	V_{tm} r	+12 2	+10 1	+6 9	−4 5	− 7 2	− 9 0
	μl c	+11 9	+10 1	+6 8	−4 6	− 7 6	− 9 2
431129 E S	V_{tm} r	+14 8	+12 7	+8 3	−6 8	− 9 8	−11 8
	μl c	+14 4	+12 2	+8 2	−6 5	− 9 8	−11 4
450313 J S	V_{tm} r	+14 4	+11 8	+7 7	−6 1	− 9 0	−11 8
	μl c	+14 2	+11 8	+7 4	−5 8	− 9 1	−11 5

The difference between the volume pressure relationship of the tympanic membrane and middle ear systems

The volume pressure relationship of the tympanic membrane system $V_{tm}=f(P_{tm})$ is recorded when known pressures in the rhinopharynx are propagated quantitatively to the middle ear by deglutition The pressure in the ear canal should be constant If this pressure is changed — as during recording of the volume pressure rela tionship of the middle ear system $V_{tm}=f(P\ )$ — the middle ear pressure is not constant During the pressure change in the ear canal there is a change of the middle ear volume ΔV_m caused by the displacement of the drum ΔV_{tm} The resulting change in the pressure ΔP_m can be calculated from Boyle s law

$$\Delta P_m = \frac{\Delta V_{tm}}{V_m}(P_{atm} - P_{H_2O})$$

Owing to ΔP_m the volume pressure relationship of the tympanic membrane system differs from that of the middle ear It is evident from the equation above that there are two factors affecting this difference (*a*) ΔV_{tm} which is related to the mobility of the tympanic mem brane (*b*) The volume of the middle ear

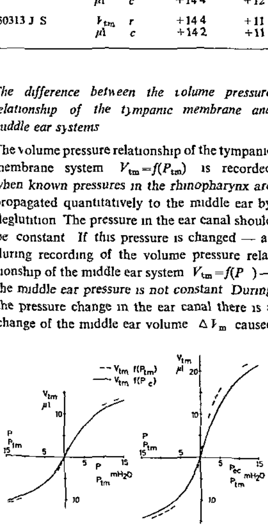


Fig 3 Comparison between the static volume pressure relationships of the tympanic and middle ear systems Left normal drum and middle ear volume of 6 ml Right flaccid drum and middle ear volume of 6 ml

In Fig 3 the two volume pressure relationships are compared first in a case with a normally movable drum and then in a case with a flaccid drum The middle ear volumes are in both cases 6 ml

As shown by Fig 3 the difference between $V_{tm}=f(P_{tm})$ and $V_{tm}=f(P\ )$ is very small when the drum is normally movable but increases when it is flaccid These two examples have further been analysed to show how different middle ear volumes affect this difference It is apparent from Table II that the volume pressure rela tionships are nearly identical at a middle ear volume of 6 ml or more whereas they differ at middle ear volumes smaller than 6 ml especially in cases with flaccid drums

Table II *Comparison of the difference between the volume pressure relationship of the tympanic and middle ear systems $V_{tm}=f(P_{tm})$ and $V_{tm}=f(P_{ec})$ in cases with normal and flaccid drums and different volumes of the middle ear*

$+P_{tm}$ $-P_{ec}$ cm H_2O	$V_{tm}=f(P_{tm})$ µl	$V_{tm}=f(P_{ec})$ (µl)		
		V_m 10 ml	V_m 6 ml	V_m 2 ml
Normally movable drum				
+15	+15 0	+14 5	+14 2	+12.1
+10	+13 0	+12.3	+11 8	+ 9 3
+ 5	+ 8 8	+ 7 8	+ 7 3	+ 5 0
− 5	− 6 5	− 6 2	− 5 8	4 6
−10	− 9 3	− 8 8	− 8.5	− 7 4
−15	−11 0	−10 6	−10 3	− 9.2
Flaccid drum				
+15	+25 4	+24.2	+23 3	+17 7
+10	+22.6	+20 6	+19 3	+13 2
+ 5	+16 2	+13 5	+12.0	+ 7 3
− 5	−11 4	−10 4	− 9.5	− 6 4
−10	−15 1	−14.3	−13 6	−11 1
−15	−17 4	−16 6	−16 0	−13 7

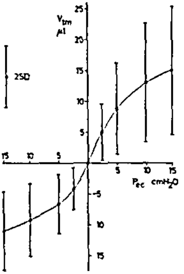


Fig 4 Mean curve of the volume pressure relationship of the middle ear system $V_{tm}=f(P_{ec})$ on 101 subjects with normal ears

The elastic properties of the middle ear system in 101 subjects

Material The material consists of subjects with normal ears recruited among blood donors and medical students There was a negative history of ear trouble and a normal status at ear examination in all cases The drum was normally movable in Siegle s otoscope The hearing threshold in all audiograms was within 0–20 dB (related to ISO standard 1964) All persons were without signs of catarrhal infection on examination The tubal function of these 101 subjects has been studied (Elner et al 1971*b*) 73 subjects could equilibrate relative over and underpressure completely (group I) Of these 33 equilibrated completely by 1–3 deglutitions (group Ib) 37 by 4–10 deglutitions (group Ic) while the rest (3 cases) had a patulous tube 21 cases equilibrated with small residual pressures (group II) 2 cases could equilibrate relative overpressure but not underpressure (group III) and 5 cases finally were completely unable to equilibrate any pressure by deglutition (group IV)

Results The volume pressure relation of the middle ear system under static pressure conditions $V_{tm}=f(P_*)$ has been determined on 101 persons and the neutral position of the drum has been calculated with the aid of the mean value deviation for Pch_{rmax} On each volume pressure diagram V_{tm} has been calculated at P_e

Table III *Volume pressure relationship of the middle ear system $V_{tm}=f(P\ )$ in 101 subjects with normal ears*

The neutral position of the ear drum is calculated from the m an valu^ deviation of Pch_{max} when the drum moves inwards

V_{tm} µl	P_{ec}, cm H O							
	−15	−10	−5	−2.5	+2.5	+5	+10	+15
Mean	+15 0	+13 0	+8 8	+5 0	−4 1	−6 5	− 9 3	−11 0
S D	± 5 2	± 4 8	±3 7	±2 3	±1 7	±2 4	± 2.9	± 3 2
S E	± 0 5	± 0 5	±0 4	±0 2	±0 2	±0 2	± 0 3	± 0 3

Table IV *Volume pressure relationship of the middle ear system $V_{tm}=f(P_{ec})$ in the different tubal function groups*

The neutral position is calculated from the mean value deviation of Pch_{Fmax} when the drum moves inwards

Tubal function group (No of subjects)	V_{tm} µl	P_{ec} cm H O −15	−10	−5	−2.5	+2 5	+5	+10	+15
I (73)	Mean	+13 3	+11 5	+ 7 7	+4 5	−3 6	−5 8	− 8.5	−10 1
	SD	± 3 5	± 3 3	± 2.4	±1 7	±1 1	±1.5	± 1 9	± 2 2
	SE	± 0 4	± 0 4	± 0 3	±0.2	±0 1	±0 2	± 0 2	± 0 2
II (21)	Mean	+18 7	+16 3	+11 1	+6 3	−5 0	−7 9	−10 9	−12 9
	SD	± 6 4	± 5 8	± 4 5	±2 9	±2 2	±3 2	± 3 8	± 4 2
	SE	± 1 4	± 1 2	± 1 0	±0 6	±0 5	±0 7	± 0 8	± 0 9
III IV (7)	Mean	+21 1	+18 5	+13 3	+8 0	−6 0	−9 1	−12 3	−14.2
	SD	± 7 1	± 7 0	± 5 1	±3 7	±2.2	±3 0	± 3 5	± 3 6
	SE	± 2 7	± 2 6	± 2 3	±1 4	±0 8	±1.2	± 1 3	± 1 4

±2 5 ±5 ±10 and ±15 cm H_2O (Table III) On the basis of the mean values a mean curve of the volume pressure relationship of the middle ear system has been constructed (Fig 4)

The volume pressure relationship of the middle ear system $V_{tm}=f(P_{ec})$ in relation to the tubal function has been determined for tubal function groups I II and III-IV (Table IV)

Furthermore $V_{tm}=f(P\ )$ in tubal function group Ib and Ic are compared (Table V)

Two or three repeated determinations of the volume pressure relationship of the middle ear system have been made on 13 cases The interval is several months There is good agreement between these values on the different occasions The error of the method based upon these repeated recordings has been calculated according to the R method (see statistical note page 7) and amounts to less than 10

DISCUSSION

A direct determination of the volume pressure relationship of the tympanic membrane system $V_{tm}=f(P_{tm})$ can only be made on subjects with a perfect tubal function In other cases only the volume pressure relationship of the middle ear system $V_{tm}=f(P_{ec})$ can be recorded This however provides good information about the tympanic membrane system as the difference is very small in cases with a normally movable drum and normal middle ear volume The difference is greater however in cases with flaccid drums and small volumes In these latter cases the difference can be reduced by correction of the pressure change in the middle ear caused by the tympanic membrane deviation

In the 101 cases with normal ears the volume pressure relationship of the middle ear system

Table V *Volume pressure relationship of the middle ear system $V_{tm}=f(P_{ec})$ for the tubal function groups Ib and Ic*

The neutral position calculated from individual Pch_{Fmax} when the drum moves inwards

Tubal function group	V_{tm} (µl)	P_{ec} cm H O −15	−10	−5	−2.5	+2.5	+5	+10	+15
Ib (33 subjects)	Mean	+1[illegible]	+10 4	+7 0	+4 0	−3 0	−5 0	− 7 3	− 8 9
	SD	± 2 6	± 2 4	±1 8	±1 2	±0 9	±1 3	± 1 7	± 1 9
	SE	± 0 5	± 0 4	±0 3	±0.2	±0 1	±0.2	± 0 3	± 0 3
Ic (37 subjects)	Mean	+14 4	+12 7	+8 6	+4 9	−4 1	−6 7	− 9 6	−11 4
	SD	± 3 6	± 3 3	±2 6	±1 7	±1 4	±2 2	± 2 8	± 3 1
	SE	± 0 6	± 0 5	±0 4	±0 3	±0 2	±0 4	± 0 5	± 0.5

under static pressure conditions varies considerably (Table III) On the other hand there is a very small variation in each person at different times which appears from the repeated determinations with an interval of several months

A comparison between the volume–pressure relationships of the middle ear system in the different tubal function groups has been made Statistically there is a highly significant difference ($p < 0.001$) between the volume pressure relationship in group I b and I c between group I and II and between group I and III–IV There is no significant difference between group II and III–IV

If a person is not subjected to changes in the ambient pressure the Eustachian tube if functioning normally ought to equilibrate a relative underpressure in the middle ear caused by absorption of oxygen and nitrogen from the ear The degree of normal function of the tube varies even among persons with completely normal ears In cases with perfect tubal function group I the middle ear pressure is kept very near to the ambient pressure However the middle ear pressure differs more from the ambient pressure in cases with a less good tubal function groups II III and IV (Elner et al 1971 *b*) and the drum is probably subjected to greater strain in these cases In support of this assumption we find significant differences in the volume pressure relationship of the middle ear system between the different tubal function groups and the mobility of the drum has a tendency to increase with a reduced tubal function (Table IV) Short term experimental pressure loads of ± 15 cm H_2O however do not affect the mobility of the drum On the other hand the volume pressure relationship of the middle ear system changes at greater pressure differences across the drum i e by fast changes of the ambient pressure up to 100 cm H_2O These high pressure levels are therefore never used In contrast to the volume pressure relationship of the middle ear system under static pressure conditions there is a hysteresis in the recording under dynamic pressure conditions

The recording in Fig 2 shows how the volume pressure curve of the middle ear system follows different tracks when the pressure decreases and increases during the change of the ambient pressure At these recordings under dynamic pressure conditions we got similar volume pressure curves in all cases but with a slight variation in hysteresis The cause of this hysteresis is being studied The result will be published later

ZUSAMMENFASSUNG

Die Verfasser haben eine Methode entwickelt zur Registrierung der Volumenabweichung des Trommelfells bei verschlossenem Mittelohr als einer Funktion der Druckveränderung im äusseren Gehörgang Von diesen Registrierungen können die elastischen Eigenschaften des Trommelfellsystems berechnet werden Studien dieser Art sind bis jetzt nicht veroffentlicht worden In 101 Fällen mit normalen Ohren fanden wir eine Trommelfellbeweglichkeit von wechselndem Ausmass Die Beweglichkeit ist in Fallen mit leicht beschränkter Tubenfunktion erhoht im Vergleich zu vorzuglich funktionierenden Fällen Dieses Resultat erklärt sich durch eine erhöhte Druckbelastung auf das Trommelfellsystem wenn die Druckausgleichsfahigkeit der Tube beeinträchtigt ist Die dargelegte Methode erscheint uns zum Studium pathologischer Veranderungen im Mittelohr sehr geeignet

STATISTICAL NOTE

The *R* method has been used for calculation of the error of the method

$$S = \frac{R_1 + R \quad R_{13}}{11\alpha_2 + 2\alpha_3}$$

where *R* is the difference between the highest and lowest values at repeated recordings on one and the same person and *S* is a value which if calculated as of the mean value of all the V_{tm} values gives the error of the method $\alpha_2 = 1\,128$ $\alpha_3 = 1\,693$

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DIRECTIONAL AUDIOMETRY

III The Influence of Azimuth on the Perception of Speech in Patients with Monaural Hearing Loss

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Abstract The Directional Threshold of Intelligibility (DTI) both with and without background noise has been determined in 37 patients with on sided hear ing defects ranging from a 30 dB loss to total deaf ness Free field speech audiometry was done with an apparatus permitting independent radial position ing of a speech loudspeaker and a noise loudspeaker on the circumference of a horizontal circle centred on the head of the seated person examined The DTI measured without noise not only depended upon the amount of the hearing loss in the d fective ear but also upon slight variations in the hearing ability of th good ear A relationship between the DTI with out noise and the normal ear s Pure Tone Average (PTA) in the range 500 1 000 and 2 000 Hz was found In the persons examined it was also found that when speech was reproduced against background noise under certain conditions the bad ear con tributed to the perception of speech if the person test d had one sided PTA of 53 dB hearing level or less

It has long been known that we have one organ of hearing with two holes Hirsh (1948) gave a historical review of investiga tions and speculations regarding one side of this question in his article Binaural Sum mation—A Century of Investigation Rey- nolds & Stevens (1960) also dealt with the bi naural interaction and the neurophysiologi cal connections between the two ears have been explored (e g Galambos et al 1959)

That two ears give better intelligibility of speech than one is especially marked when the speech is presented against background noise (Bocca 1955, Bergman 1957 Pollack & Pickett 1958 Groen & Hellema 1960 Chappell et al 1963 Carhart 1965 Harris 1965)

Egan (1948) found that even though in tense noise in one ear masked speech in the other ear a weaker noise improved the in telligibility Licklider (1948) demonstrated that speech intelligibility was improved for mon aurally presented words mixed with noise when identical noise was presented to the opposite ear This was confirmed by Weston et al (1965)

Persons with one sided loss of hearing may therefore be expected to be handicapped com pared to normal hearing individuals This is confirmed by the works of Wark (1967) and Giolas & Wark (1967) The present work has been done to investigate the hearing problems of this special group with one sided loss of hearing

INVESTIGATIONS

In a previous publication (Tonning 1970) an apparatus for the measurement of the Direc tional Threshold of Intelligibility (DTI) has been described and in another (Tonning in press) the DTI of 30 normally hearing sub

Table I *The relationship between British Standard 1954 and ISO Standard 1964 for our equipment (earphone TDH 39/MX41AR 9A coupler)*

The table indicates the number of dB that must be added to the threshold of hearing recorded in British Standard when transferring to ISO Standard

Frequency Hz	125	250	500	1000	2000	4000	6000	8000
dB added to hearing loss in British Standard when transferring to ISO Standard	+3 5	+3 7	+2 0	+2 9	−0.2	0 0	+7 9	−5 5

jects has been measured In order to determine the influence of monaural hearing loss on the perception of spatially oriented speech the DTI of a group of patients with one sided loss of hearing was determined both with and without background noise using the procedure described previously (op cit) (Throughout the following the dB reference level is 0 0002 dyne per square centimetre Hearing loss in dB refers to British Standard 2697 1954 see also Whittle & Robinson 1961) The relationship between ISO standard 1964 and British Standard 1954 for our equipment is shown in Table I (Whittle & Delany 1966)

In 37 patients with monaural hearing impairment the Directional Threshold of Intelligibility (DTI) was recorded with the signal loudspeaker in four different positions (0 = in front 90 = to the right 180 = behind 270 = on the left side of the person tested) The same experiment was then made with background noise (65 dB white noise) for each of the four positions of the signal loudspeaker four positions (0 90 180 and 270) of the white noise source were used giving 16 different combinations of positions of the signal and noise loudspeakers

The dB value of DTI refers to the intensity at the point 0 9 metre from the signal loudspeaker This point corresponds to the centre of the head of the person examined Without any person in the sound field these values were controlled by calibration prior to the actual testing

By selecting subjects with hearing loss only in one ear it should be possible to investigate some of the correlations between the functions of the two ears in the perception of speech We also wanted to get information about the amount of hearing loss at which the deaf ear ceases to function as a receiver of the speech stimulus under these experimental conditions

MATERIAL

37 right handed persons were examined 11 women and 26 men The age varied from 12 to 65 years The average age was 41 years which also was the median age of the sample Pure tone audiograms were recorded for the frequencies 125 250 500 1 000 2 000 4 000 6 000 and 8 000 Hz using a Madsen Electronics Audiometer Model OB 60 calibrated according to British Standards The hearing loss occurred on the right side in 18 cases and on the left side in the remaining 19 cases All of these patients had normal hearing in the other ear i e thresholds better than 20 dB hearing level for all the frequencies tested For the pathological ear the hearing loss is indicated by the Pure Tone Average (PTA) for the frequencies 500 1 000 and 2 000 Hz To avoid very steep audiograms persons having a bad ear with an individual difference of more than 30 dB between the lowest and highest threshold at these frequencies were not accepted

In Table II the 37 subjects tested are listed in the order of increasing hearing loss In 10 cases there was no measurable hearing in the pathological ear two of these (patients 18 and 37) have been operated for acoustic neuroma

RESULTS

Influence of unilateral hearing impairment on perception of speech in free field without background noise

The results of the DTI recordings without white noise are tabulated in Table III For the sake of comparison the arithmetic mean of DTI ($\overline{DTI}$) and the standard deviation (s) are given together with the values for $\overline{DTI}$ and s for 30 normal hearing persons taken from a previous investigation (Tonning in press)

The figures seem to indicate that there is a greater dispersion in the values recorded for the monaural hard of hearing than for the persons with normal hearing

For each of the 4 positions of the signal loudspeaker we compared the DTI values of our 37 patients with those of the 30 per sons with normal hearing examined earlier (Tonning 1970 in press) For all of the 4 positions higher DTI values were found for one sided hard of hearing patients than for the 30 normal hearing persons examined earlier

(Statistics we presumed the cumulative functions of probability for the two selections continuous and in creasing in their respective areas of variations We used on sided Mann Whitney Test level of sig nificance 0 05 References Siegel (1956) Electronic computer analysis was used The calculations in this article have been done according to IBM System 360 Scientific Subroutine Packag (360A CM 03X) Version III 1968)

As expected the best DTI value for our 37 patients was found with the signal loudspeaker facing the good ear The poorest value was found with the loudspeaker aiming at the bad ear With the signal loudspeaker in front of or behind the test persons DTI results were found lying between these extreme values

(Statistics we presumed the differences x x of the coupled observations independent and identically distributed with a cumulative function of probability F_t this continuous and increasing in th variation area of x Wilcoxon Matched Pair Signed Rank Test level of significanc 0 05 was used according to Dixon & Massey (1957) Electronic computer was used)

Table II *Patients with unilateral hearing loss Patients 1–18 have impaired hearing in the right ear nos 19–37 in the left ear*

PTA Average hearing level at 500 1 000 and 2 000 Hz.
Cond Conductive hearing loss
Sn Sensorineural hearing loss
Mixed Combination of conductive and sensorineural hearing loss
Re Residual hearing Hearing not measurable at all frequencies tested
No No measurable hearing
Fowler test + indicates recruitment – indicates no recruitment No sign indicates that the test has not been done

Pat No	PTA right ear	PTA left ear	Type hearing loss	Fowler test	Sex	Age (years)
1	30	1 7	Mixed	+	♂	55
2	33	6 7	Sn	+	♀	53
3	36	8 3	Mixed	–	♀	6?
4	45	0 0	Comb	–	o	3?
5	45	3 3	Cond		o	23
6	45	10 0	Mixed	–	♂	39
7	55	0 0	Sn	+	o	19
8	55	6 7	Mixed	–	♀	44
9	85	3 3	Sn		o	43
10	Re 80	0 0	Sn		o	32
11	Re 105	0 0	Sn		♀	18
12	Re	8 3	Sn		♂	27
13	Re	10 0	Sn		♂	43
14	Re	16 7	Sn		♀	52
15	No	11 7			♂	40
16	No	0 0			♀	2
17	No	13 3			♀	41
18	No	1 7	Sn		♂	28
19	3 3	32	Sn	+	♂	61
20	5 0	47	Cond		♀	47
21	10 0	53	Sn	+	♂	36
22	10 0	53	Sn	–	♂	61
23	6 7	55	Mixed	–	♂	4?
24	1 7	60	Sn	+	♂	12
25	11 7	60	Sn	+	o	53
26	1 7	65	Mixed	–	♀	37
27	8 3	70	Mixed	–	♂	65
28	3 3	80	Mixed		o	59
29	3 3	Re 85	Mixed		o	63
30	1 7	Re	Sn		♀	70
31	3 3	Re	Sn		o	35
32	5 0	No			o	39
33	11 7	No			♀	49
34	3 3	No			♂	24
35	11 7	No			♂	53
36	6 7	No			o	53
37	8 3	No	Sn		♂	26

The poorest listening position without noise was found with the loudspeaker facing the bad ear Thus it seems reasonable to propose the hypothesis that a relation exists between DTI for this loudspeaker position and the

Table III *Directional threshold of intelligibility without background noise for 37 patients with monaural hearing loss*

For each position of the signal loudspeaker the arithmetical mean of DTI ($\overline{DTI}$) and the variance (s) are calculated For the sake of comparison the values recorded in 30 subjects with normal hearing (Tonning 1970 in press) are listed

Position of signal loudspeaker	Right ear bad	Left ear bad	Normal ears
In front	$\overline{DTI}$ = 21 1 s = 3 34	$\overline{DTI}$ = 23 0 s = 4 03	$\overline{DTI}$ = 15 6 s 1 63
Right	$\overline{DTI}$ = 24 0 s = 3 43	$\overline{DTI}$ = 20 1 s = 3 36	$\overline{DTI}$ - 13 8 s 1 77
Behind	$\overline{DTI}$ = 21 9 s = 4 31	$\overline{DTI}$ = 23 4 s = 4 46	$\overline{DTI}$ - 16 0 s = 1 46
Left	$\overline{DTI}$ = 18 7 s = 3 77	$\overline{DTI}$ = 26 1 s = 4 67	$\overline{DTI}$ = 13 8 s = 1 98

PTA of the bad ear Using Spearman s Rank Correlation Coefficient RS we avoided the assumption of bivariate normality when testing RS = 0 against RS ≠ 0 Using two-sided test level of significance 0 05 we rejected the hypothesis that a connection existed Electronic computer analysis was used

The same test was made analogously on the hypothesis of a connection existing between the PTA of the good ear and the DTI observed with the signal loudspeaker facing the bad ear When calculating in this way we found that this hypothesis was not to be rejected On this foundation it was concluded that there was a relationship between the threshold for pure tones of the good ear and the DTI recorded without noise A study of the statistical calculations referred to led to the conclusion that one sided hearing loss entails asymmetric hearing in silence represented through asymmetric DTI values

However when DTI determinations are made under our conditions in a quiet field speech will be perceived by the normal hearing ear no matter what the position of the signal loudspeaker Pollack (1948) used ear phones and determined the monaural and binaural threshold for white noise and for a tone of 1 000 Hz. It was found that the binaural threshold (each ear stimulated at the same intensity level) may not appear to be statistically significantly different from the threshold of the better ear with observers in whom the difference between the two monaural thresholds is greater than 6 dB Thus the measurement of DTI in silence for the purpose of investigating the effect of an asymmetric hearing loss on the perception of speech turned out to give insufficient information The experiments were therefore repeated with a simultaneous stimulation of the test subject with an interference sound white noise

Influence of unilateral hearing impairment on perception of speech in free field with background noise

In this second series of tests measurements of the DTI values for the 37 monaural hard of hearing subjects were performed during simultaneous stimulation with white noise The signal loudspeaker was placed in each of the previously mentioned four positions in turn For each of these positions a noise loudspeaker was also placed in the same four positions resulting in a total of 16 different test combinations In Table IV the mean of the DTI ($\overline{DTI}$) and the standard deviation (s) are given for each combination of the loudspeakers For comparison the values for $\overline{DTI}$ and s for the 30 normal hearing persons examined earlier (Tonning in press) are tabulated A comparison of the variance of $\overline{DTI}$ without noise with the variance of $\overline{DTI}$ in noise is difficult as analysis of the variance is also sensitive to displacement from the median

The best listening position was found with the signal loudspeaker aiming at the good ear and the noise loudspeaker facing the bad ear The poorest listening position was found with the signal loudspeaker facing the bad ear and the noise loudspeaker aiming at the good ear The DTI for each of the 16 combinations of the signal loudspeaker and noise loudspeaker for the 37 patients was compared with

Table IV *Directional threshold of intelligibility with background noise for 37 patients with monaural hearing loss*

For each position of the signal loudspeaker the arithmetical mean of DTI ($\overline{DTI}$) and the variance (*s*) are calculated For the sake of comparison the values recorded in 30 subjects with normal hearing (Tonning 1970 in press) are listed

Position of signal loudspeaker	Right ear bad	Left ear bad	Normal ears
Position of noise loudspeaker in front			
In front	$\overline{DTI}$ = 53 3	$\overline{DTI}$ = 53 4	$\overline{DTI}$ = 51 0
	s = 2 05	*s* = 1 43	*s* = 1 58
Right	$\overline{DTI}$ = 55 3	$\overline{DTI}$ = 49 4	$\overline{DTI}$ = 43 6
	s = 3 09	*s* = 1 83	*s* = 2 25
Behind	$\overline{DTI}$ = 53 3	$\overline{DTI}$ = 53 1	$\overline{DTI}$ = 50 0
	s = 2 35	*s* = 1 75	*a* = 1 87
Left	$\overline{DTI}$ = 49 9	$\overline{DTI}$ = 54 8	$\overline{DTI}$ = 43 5
	s = 2 45	*s* = 2 99	*s* = 2 06
Position of noise loudspeaker right ear			
In front	$\overline{DTI}$ = 50 4	$\overline{DTI}$ = 54 5	$\overline{DTI}$ = 45 3
	s = 2 20	*s* = 2 69	*s* = 1 90
Right	$\overline{DTI}$ = 54 8	$\overline{DTI}$ = 52 9	$\overline{DTI}$ = 50 8
	s = 2 62	*s* = 2 31	*s* = 1 65
Behind	$\overline{DTI}$ = 51 2	$\overline{DTI}$ = 55 2	$\overline{DTI}$ = 46 2
	s = 2 13	*s* = 2 07	*s* = 1 85
Left	$\overline{DTI}$ = 47 7	$\overline{DTI}$ = 56 6	$\overline{DTI}$ = 43 4
	s = 2 19	*s* = 3 64	*s* = 1 87
Position of noise loudspeaker behind			
In front	$\overline{DTI}$ = 51 4	$\overline{DTI}$ = 52 2	$\overline{DTI}$ = 49 4
	s = 1 65	*s* = 2 06	*s* = 1 77
Right	$\overline{DTI}$ = 53 9	$\overline{DTI}$ = 48 6	$\overline{DTI}$ = 43 2
	s = 2 52	*s* = 2 32	*s* = 2 37
Behind	$\overline{DTI}$ = 52 8	$\overline{DTI}$ = 53 5	$\overline{DTI}$ = 50 5
	s = 1 48	*s* = 1 93	*s* = 1 89
Left	$\overline{DTI}$ = 48 8	$\overline{DTI}$ = 54 4	$\overline{DTI}$ = 42 8
	s = 1 86	*s* = 2 71	*s* = 2 47
Position of noise loudspeaker left ear			
In front	$\overline{DTI}$ = 54 6	$\overline{DTI}$ = 49 8	$\overline{DTI}$ = 45 1
	s = 1 98	*s* = 2 12	*s* = 2 07
Right	$\overline{DTI}$ = 56 6	$\overline{DTI}$ = 47 1	$\overline{DTI}$ = 43 4
	s = 3 14	*s* = 2 02	*s* = 2 10
Behind	$\overline{DTI}$ = 55 4	$\overline{DTI}$ = 51 1	$\overline{DTI}$ = 46 2
	s = 2 92	*s* = 1 65	*s* = 1 86
Left	$\overline{DTI}$ = 53 3	$\overline{DTI}$ = 54 2	$\overline{DTI}$ = 50 8
	s = 1 68	*s* = 1 84	*s* = 1 75

the results for the 30 normal hearing persons The hypothesis of equal DTI values for the same listening positions for the two samples was rejected for all positions indicating higher DTI values for the monaural hard of hearing patients than for the normal hearing persons

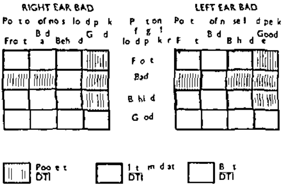


Fig 1 Diagram showing the 5 poorest the 5 best, and the 6 intermediate listening positions for 18 patients with impaired hearing in the right ear and 19 with the left ear impaired

(Statistics we presumed the cumulative functions of probability for the two selections continuous and increasing in their respective areas of variations One sided Mann Whitney Test was used level of significance 0 05 Electronic computer was used)

In Fig 1 the $\overline{DTI}$ for the patient group with right sided hearing loss and that with left sided loss are arranged separately in order according to their numerical values The 5 poorest the 5 best and the 6 intermediate listening positions for these two groups are indicated

Illustrated in this way we observe a tendency to symmetry the five best listening conditions for the right sided deaf patients are the same as for the left sided deaf persons The five poorest listening conditions for the right sided deaf persons nearly correspond to those of the left sided deaf subjects The six intermediate listening conditions are nearly the same for both groups

Thus we find a conformity on many points between these two groups in respect of the $\overline{DTI}$ in noise However there is a wide variation in hearing in the bad ear from one person to another in each of these groups It therefore seems reasonable to investigate whether the degree of hearing loss in the bad ear is of importance for the discrimination of

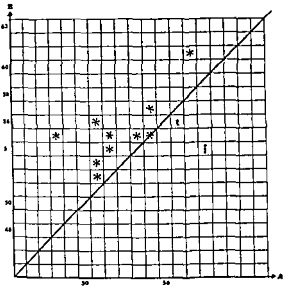


Fig 2 Scattergram showing the relationship between th PTA of the bad ear and the DTI *Abscissa* DTI values observed with the signal loudspeaker aiming at the bad ear noise loudsp aker in the contralateral position *Ordinate* DTI values observed with signal and noise loudspeakers aimed at the bad ear *PTA < 53 dB •PTA ≥ 55 dB

speech in noise To evaluate this we reason as follows Let us consider two experimental conditions A and B

Condition A

the signal loudspeaker aiming at the bad ear the noise loudspeaker aiming at the good ear

Condition B

both loudspeakers facing the bad ear

If the bad ear is of importance for the discrimination of speech in noise we will expect lower (better) DTI values under condition A than under condition B If the bad ear is of no essential importance for the discrimination of speech in noise we will expect lower (better) DTI values under condition B than A In Fig 2 the DTI results for our 37 patients are plotted in a coordinate system The abscissa indicates the values of DTI under condition A The ordinate indicates the values found under condition B Identical values for the two conditions would thus be lying on the oblique line drawn in the coordinate system forming an angle of 45 with the axes If the bad ear contributes significantly to the discrimination of speech in noise we will get points lying in the coordinate system above the oblique line If the bad ear is of no essential importance for the discrimination we will expect points lying the coordinate system below the oblique line

As can be seen from the figure we get points lying above as well as on and below the oblique line The points lying on and above this line belong to patients with a PTA of 53 dB hearing level or less while the points below the line belong to persons with PTA of 55 dB hearing level or more This leads to the conclusion that in the patients with a monaural PTA of 53 dB hearing level or less the bad ear contributes to the perception of speech under our mentioned experimental conditions (white noise of 65 dB) If the PTA is 55 dB hearing level or more this bad ear does not contribute to the perception of speech under our experimental conditions

Use of the standard diagram

In order to illustrate how the DTI values of the test group compare to normally hearing standard subjects under the last described experimental conditions the standard diagram developed in a previous article (Tonning 1971) is used (see Fig 3)

To make it easier to understand the diagram we can plot the DTI values of a normally hearing person in the coordinate system

1 Signal loudspeaker aimed at the left ear noise loudspeaker at the right ear

DTI without noise

14 dB

DTI with noise

43 dB Point plotted at A

2 Both loudspeakers aimed at the left ear

DTI without noise

14 dB

DTI with noise

50 dB Point plotted at B

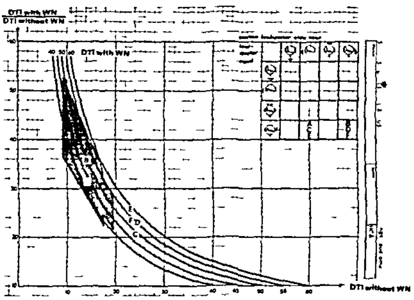


Fig 3 Coordinate syst m where the shaded polygon covers 95 of the DTI values for 30 normal hearing persons examined earlier (Tonning in press) In this coordinate system some DTI values are plotted for 1) a person with normal hearing (Points A and B) 2) a person with l ft sided hearing lo s of TC<53 dB (Points C and D) 3) a person with left sided hearing lo s of TC≥55 dB (Points E and F) The actual experimental listening conditions are indicated on the figure in the upper right corner of the coordinate system For furth r explanation see text

The standard diagram shows that Point B is lying on iso line above the level of Point A expressing the greater masking effect of the oise in the latter test position

In patient 19 having a PTA of 32 dB hearing level in the left ear one finds

1 Signal loudspeaker aimed at the left ear noise loudspeaker at the right ear
DTI without noise
24 dB
DTI with noise
48 dB Point plotted at C

2 Both loudspeakers aimed at the left ear
DTI without noise
24 dB
DTI with noise
55 dB Point plotted at D

Point D lies on iso line above the level of Point C indicating that the left pathological ear took part in the perception of speech against background noise The fact that these points are lying to the right of the area of the normal hearing standard group indicates that the subject s hearing without background noise is not so good as that of the normally hearing standard group The iso lines through the points cut the area of the standard group indicating that the patient s perception of speech in noise under these conditions corresponds to the values of the standard group

In patient 29 having a PTA of 85 dB hear*ing level in the left ear one finds*

1 Signal loudspeaker aimed at the left ear noise loudspeaker at the right ear
DTI without noise
23 dB
DTI with noise
59 dB Point plotted at E

2 Both loudspeakers aimed at the left ear
DTI without noise

23 dB
DTI with noise
53 dB Point plotted at F

The standard diagram shows that Point F is lying on iso line at a lower level than Point E indicating that under these experimental conditions the good ear contributed to the subject's perception of speech

COMMENTS

Our investigations were made in an anechoic room The directional effect of the noise was more pronounced here than it would be in our ordinary daily surroundings The noise offered was not noise usually occurring in everyday life either The investigation of speech perception took place at the threshold Thus our experimental listening situation differed considerably from a real hearing situation in everyday life

Under our experimental conditions without noise speech was perceived by the good ear Under certain experimental conditions described in the article the bad ear contributed significantly to the speech discrimination when the PTA $\leqslant$53 dB hearing level Under the same experimental conditions this was not true when the hearing loss was PTA $\geqslant$55 dB hearing level

ZUSAMMENFASSUNG

Die gerichtete Verständlichkeitsschwelle (Directional Threshold of Intelligibility—DTI) sowohl mit als auch ohne Hintergrundgeräusch wurde bei 37 Patienten mit einseitigen Hörschaden bestimmt, und zwar für Hörverluste von 30 dB bis zu völliger Taubheit. Frei Feld Sprachaudiometrie wurde mit einem Gerät vorgenommen das eine unabhängige radiale Aufstellung je eines Sprach Lautsprechers und eines Geräusch Lautsprechers am Umkreis eines horizontalen Kreises mit dem Kopf des sitzenden Versuchsobjektes als Zentrum gestattet. Die ohne Geräusch gemessene Schwelle war nicht nur von der Höhe des Hörverlustes im geschädigten Ohr abhängig sondern auch von geringen Variationen im Hörvermögen des gesunden Ohres Man fand einen Zusammenhang zwischen der DTI ohne Geräusch und dem durchschnittlichen Hörvermögen des gesunden Ohres für die Frequenzen 500 1 000 und 2 000 Hz (= PTA. Pure Tone Average) Bei den untersuchten Personen fand man auch dass Sprache welche gegen Hintergrund geräusch hervorgebracht wurde unter bestimmten Verhältnissen vom schlechten Ohr einer Person mit einseitigem Hörverlust von PTA 53 dB oder weniger vernommen werden konnte

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Acta Otolaryng 72 413–420 1971

VIEWPOINTS ON THE MANAGEMENT OF RETROCOCHLEAR LESIONS IN CONNECTION WITH A CASE REPORT

A Tumour Originating from the Glossopharyngeal Nerve

T Lundborg

From the Department of Audiology Sodersjukhuset Stockholm Sweden

(Received March 30 1971)

Abstract In 1949 Olivecrona stated that further progress in the management of acoustic tumours could be expected only after progress in the ability of non-neurosurgeons to recognize early tumours Thus a medical problem was faced which was rather well solved in its neurosurgical aspects, yet to otologists remained a challenge to clinical research The author s work revealed that it was possible for the otologist to consider the *overall picture* of these cases. The next step was the development of hearing tests to differentiate the hearing loss concerning site of lesion. A further step was the development of otomicroscopic surgical approaches to the inner meatus Otology and neurosurgery have thus been characterized by the development of the management of acoustic tumours with continuously increasing responsibility for the otologist The next step of development is expected within otology and audiology the creation of diagnostic methods, making it possible to detect the early tumour situated within the inner meatus and thus favorable to extirpation

Further viewpoints on the diagnosis of retrocochlear lesions may seem rather superfluous if one considers the proliferation of informative reports particularly during the last decade

Nevertheless in connection with a case report of a pontile angle tumour originating from the glossopharyngeal nerve it seems worthwhile recapitulating the varying standpoints in the management and treatment of retrocochlear deafness cases since many problems in these respects have been faced

Symptomatology and diagnosis of pontile angle tumours from the otologic point of view

The fact that only ca. 10% of Olivecrona s cases of acoustic tumours were diagnosed and admitted to hospital by otologists became this author's incentive to study these problems (Lundborg 1950)

It was also known from the case histories that the patients often had consulted otologists because of unilateral deafness tinnitus and vertigo This obviously constituted a challenge to clinical research An investigation of the symptomatology of the above mentioned case material thus was started with emphasis on the symptoms and examinations essential to a preliminary diagnosis and to find out if this could be established by an otologist

During that period it was considered by no means as self-evident that an otologist should play a particularly active role in the evaluation of the symptomatology in these cases Cushing s statement (1917) on the negative effect of division of responsibility was also borne in mind.

300 surgically verified cases from Olivecrona s department at Serafimerlasarettet were studied from the otologic point of view concerning the symptomatology relevant for a preliminary diagnosis It was noticed to what

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extent a basis for a preliminary diagnosis was provided by *the routine otologic examinations* (testing the hearing caloric response nystagmus corneal reflex and function of the facial nerve) and by the *special examinations* (ophthalmoscopy and x ray of porus acousticus)

The result was

(*a*) in the great majority of cases the examinations of the hearing caloric response nystagmus corneal reflex and the facial nerve provided a basis for a preliminary diagnosis

(*b*) in a relatively large group of the patients it was revealed that x ray of porus and ophthalmoscopy *complemented* the customary clinical examinations but did not replace them

(*c*) there were rather few cases with inconsistent objective symptoms unfavourable to clinical diagnosis by means of routine clinical otologic methods

(*d*) the most *frequent* objective *symptoms* were the same as the *early symptoms* (in the few cases that were diagnosed at an early stage of development) This fact had to be considered particularly favourable from the otologic point of view because a number of ıe early symptoms fell within the otologists ›here

Comment It is interesting to notice that the statement of what was considered to be the otologist s role concerning the diagnosis is in full agreement with Hitselberger s & House s conclusions and standpoints from 1968 based on their particular experience They stress that the neurologic evaluation should be carried out by otolaryngologists to establish an early diagnosis of the acoustic tumours Principally the same viewpoints were also expressed by Dix & Hallpike in 1960

End organ or nerve fibre deafness (Development of hearing tests)

Another obvious fact revealed in the investigation reported in 1952 was that the great majority of patients were deaf on admission and comparatively few cases with residua hearing were examined with pure tone audiograms

The hearing loss symptoms therefore ha to be better evaluated and thus the next ques tion arose is it possible to utilize the Bekesy audiogram in the differential diagnosis between cochlear and retrocochlear deafness? When the study was made (1950–1951) thi was one of the few audiological methods available

The question may arise why was the Bekesy audiogram (utilizing the original technique see Lundborg 1952) and no other simila hearing test exclusively used in this investigation? With the aim of extending somewha the otologist s role in the topical diagnosis o nerve deafness only up to date and efficien electro-acoustic equipment (as termed by Di & Hallpike in 1960) should be utilized

Within the institution to be sure certain other tests were already run but they wer at that time—in the author s judgement—no sufficiently well elaborated or mastered The investigation thus had to be limited to avoid inadequate test results which certainly would not have increased the diagnostic information

RESULTS

In a group of surgically verified Menieres disease patients with unilateral hearing loss (26 cases) it was revealed that almost all of the cases (24 out of 26) had small spikes at frequencies over 1 000 Hz in the Bekesy audiogram tracings i e reduced difference limen for intensity There were also a few patients with moderate reduction of the spikes

The patients with unilateral deafness with verified retrocochlear pathology did not reveal as a rule (18 out of 21 cases) reduced spikes in the Bekesy tracings but occasionally there were reduced difference limen at part of the frequency range On the other hand none of these cases revealed the typical tracings observed among the Meniere cases with highly reduced difference limen over a great part of the frequence range

Comment This investigation was made with the original Békesy audiometer using stimulation with continuous changing in 2 dB steps as compared with 0 25 dB steps in the commercially available Grason Stadler E 800 Audiometer The case material was revised concerning an eventual existence of threshold shift A few cases revealed breaks or deviation in the curves—occasionally not more found on repeated tracing—which in some way resembled type III or IV of Jerger s categorization (Jerger 1960) (Rosler personal communication 1962)

The conclusion of these findings was that *the Bekesy audiogram with reduced respectively normal spikes in the tracings* (difference limen) constituted an index of suspicion towards cochlear respectively retrocochlear lesions However this isolated symptom was not considered decisive Before more extensive experience has been gained the topographic diagnostic significance of the Bekesy audiogram should not be overestimated and particularly in cases where its appearance is not characteristic conclusions should be drawn with caution (Lundborg 1952)

Because the significance of different recruitment signs was debated it seemed necessary to study from different aspects the Bekesy tracings on further available case material concerning their topical diagnostic information

In 1960 therefore a study was made of 32 cases of end organ and 33 cases surgically verified nerve fibre deafness (Lundborg 1960) with principally the same result as in 1952

Thus the findings from the Bekesy tracings seemed to constitute a rather good basis for a biological test a fact that was also emphasized by Dix & Hallpike (1960) based on similar test results

Clinical course and morphology in acoustic tumours

Another fact noted in connection with the case study report (1952) was the evident discrepancy in time of the development of symptoms before surgery There were cases with an anamnesis of 10–20 years starting with tinnitus unilateral hearing loss and later dizziness etc

There were also cases where the same sequence of symptoms appeared during a period shorter than a year even some months

Seeking some explanation or possibly morphological differences in these two types of case a histological control was made of re*maining specimens in 30 selected cases* 15 with a short course (from 3 months to 1 year) and 15 with a long course (10–30 years) (Henschen & Lundborg 1954)

By *light microscopic observation it* was stated however that there was no morphologically visible difference in the structure of tumours of rapid and slow cases (Interesting to compare are Hitselberger s & House s remarks in 1968 that there is still need for further study of the various rates of growth of these tumours)

The lack of morphological explanation con*cerning the course and development of the* disease thus further emphasized the necessity of more meticulous diagnostic tests in otology and audiology in order better to evaluate the symptoms with the aim to make an earlier diagnosis

Hearing test battery in evaluation of nerve deafness (Modern audiology)

In the 1950s and 1960s many clinics and institutions became more interested in the evaluation of unilateral deafness using several audiological tests During this period several excellent publications were presented which in many ways helped to clarify (though not solve) the question of predicting the site of lesion *within the auditory system* In this context should be mentioned Dix & Hallpike 1960 Jerger 1961 Johnson & House 1964 Johnson 1966 1970 and others A complete list of all investigators who have made informative contributions in this connection—with some variation in test procedures results and conclusions—would be a long one

It is probably generally agreed that special credit should be given to Hallpike and co-workers (Dix et al 1948 Dix & Hallpike 1960 etc) for their classical investigations concerning end organ and nerve fibre deafness which surely became an incentive to increased activities with these cases all over the world Jerger's definitive report from 1961 (quoted from Johnson 1964) presented a new conception concerning the possibility and limitation of various hearing tests and particularly stressed the necessity to utilize an audiological test battery Through Jerger's work two new administrations of testing were developed the Békesy testing using continuous and interrupted stimulation (with Grason Stadler E 800) and the Short Increment Sensitivity Index test (SiSi test) These two methods have been widely used and constituted an essential contribution to the evaluation of sensorineural hearing loss

The experience from the Otologic Medical Group Los Angeles is reported in a series of publications (Johnson & House 1964 Johnson 1966 1970 Hitselberger & House 1968 etc) based on extensive case materials including many small lesions The meticulous study on the relationship between all possible combinations of test results (as made by Jerger 1961) is particularly informative

The important viewpoint is stressed that no *single auditory test can be considered decisive* in differentiating a retrocochlear from a cochlear lesion the full battery of auditory tests should be administered to give maximum aid (Hitselberger & House 1968) These investigations also contributed notably to an early recognition of many retrocochlear lesions thus favouring the possibilities for House and co workers to elaborate their new otomicroscopical operative approach towards these tumours

Diagnosis of recruitment or site of lesion

Part of the discussion from this period also seemed to concern primarily the presence or absence of the recruitment phenomenon or other recruitment signs more than the topical diagnosis of the lesion

From the clinical point of view the discussion would probably have been more fruitful had the tests been evaluated primarily by their topical-diagnostic information and not for their ability to demonstrate recruitment per se Already in the Dix et al classical report (1948) it was stated that in cases in which the loudness recruitment phenomenon had been observed there was a serious lack of information on the nature of the pathological process This has later been stressed particularly by Jerger (1961) pointing out that the question Is recruitment present or absent was in itself not very meaningful because of its misleading oversimplification

Similar conclusions are again drawn by Dix & Hallpike in 1960 by their statement that nothing has done more to miscredit neuro-otology than the unreasonable use in cases of this kind of over elaborate diagnostic tests

At the Fifth International Congress of Audiology in Bonn in 1960 one of the round tables concerned recruitment and recruitment tests (Hood Jerger Lundborg Konig & Wagemann) Jerger's statement mentioned above maintains that measurement of recruitment should not be the primary goal rather should one utilize a joint test result and try to discover typical test patterns The summarizing conclusion *made by Huizing (as moderator of* the panel) was also that one should not go further than that

During the last decade there has been an extensive increase in experience from evaluation of cochlear respectively retrocochlear unilateral deafness This development seems to be based partly on the work of well equipped trained and experienced audiological units A further essential incentive to increased activity in the field is a change of therapeutic attitude towards the retrocochlear lesions by introduction of otomicroscopic operative approaches the middle fossa and the translabyrinthine methods The Otologic Medical Group Los Angeles in particular (Hitselberger House

Johnson and co workers) should be credited for this development A similar oto neuro surgical cooperation to mention is Fluur & Steiner (1968) utilizing the transmeatal posterior fossa approach to the inner meatus The recent discussions concerning end organ and retrocochlear deafness also seem to reflect this development and have consequently be come primarily clinical and less speculative

The stapedius reflex test in retrocochlear lesions

An additional type of approach in the topical diagnosis of retrocochlear deafness is introduced by H Andersson (1969 and personal communication 1971) utilizing the stapedius-reflex technique Both the threshold of the stapedius reflex and the persistence of the reflex response on prolonged stimulation are noted. In a material of 21 verified cases of acoustic tumours and 8 posterior fossa tu mours—all in the early stage of development and with HT for pure tones not exceeding 60 dB and no fewer than 7 cases with completely normal HT—Andersson could distinguish two typical reactions (*a*) raised threshold to elicit stapedius reflex and (*b*) *a reflex decay* as ex pressed in more rapidly decreasing response amplitudes in time as compared with normals In the same material the results of other tests performed were consistent with anticipated test findings in only half of the cases This seems to be in agreement with Johnson s find ings in small lesions (1970)

Andersson also states that positive decay test results have been encountered in a few cases other than in tumour type retrocochlear le sions and concludes that all cases with a posi tive decay test result are suspect of retrococh lear lesion and must consequently be ruled out Further experience gained with verified case materials would confirm this technique of testing as a very useful diagnostic tool

DISCUSSION AND CONCLUSIONS

The collected experience from retrocochlear deafness cases has evidenced

1 The majority of the retrocochlear deaf ness cases show a consistent audiological test pattern though numerous retrocochlear le sions reveal inconsistent audiology test pat terns concerning one or more of the tests used The presence of unanticipated testresults there fore cannot rule out the presence of a retro-cochlear lesion Recent experience with the stapedius reflex test indicates that this proce dure may possibly constitute a safer way of screening out small retrocochlear lesions than the psychoacoustic tests Nevertheless the joint audiological test-outcome seems indis pensable and the overall picture from *all* clini cal investigations still has to be taken into consideration for the diagnosis

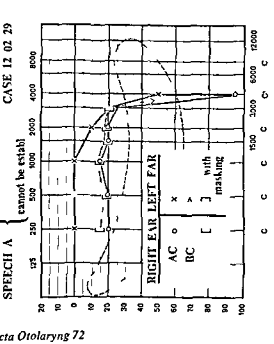
2 Since the examiner is making *observa tions* of the patient s behaviour in connection with each single test it seems realistic to note this behaviour and if possible utilize the test result clinically For various reasons a lack of agreement between the different tests is rather often revealed When new tests are introduced in the audiological test battery they have to be used on extensive case materials before their ability to predict the site of lesion can be evaluated and proven

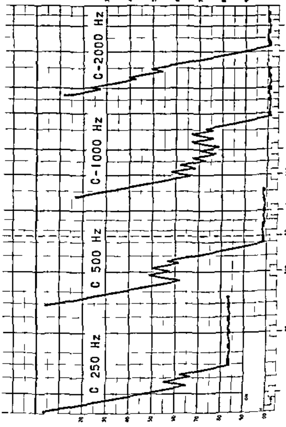
3 It is clearly evidenced that the single re cruitment of loudness symptom has no decisive topic-diagnostical significance

4 With the new otomicroscopical surgical approaches to the inner auditory canal it has become even more important to make an early diagnosis of retrocochlear deafness stressing the increased responsibility and ac tivity of otology and audiology in the field

5 Based on their particular experience on the subject Hitselberger & House (1968) con clude that patients with symptoms of uni lateral hearing loss tinnitus and vertigo single or in combination should have a complete au diologic vestibular neurologic and radiologic evaluation

With the aim of screening out the few pos sible tumour cases from the very large pa tient groups with unilateral sensorineural hear ing loss every clinical staff faces an organisa





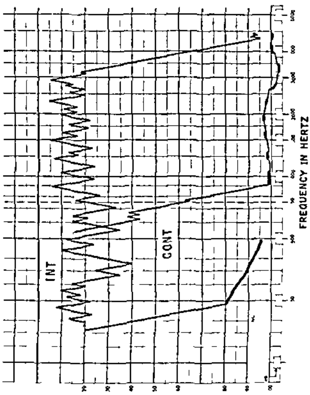


Fig 1 Case 12-03 29 Pontile angle tumour originating from the glossopharyngeal nerve right side Pure tone (and speech) audiograms Tone decay continuous tracings (C 250-2 000 Hz) Békésy audiogram with Interrupted (Int) and Continuous (Cont) tracings

tional problem A roentgen examination of the inner meatus—a very meticulous and time consuming procedure—should not for instance be utilized as a screening test in this context It is known (Greitz, 1971 personal communication) that when this examination has been carried out on wide clinical indications ca 1 000 roentgenograms of the inner meatus have been performed to find *one* positive pathological change! This is a good demonstration of the necessity to screen out some of the patients on clinical grounds i e the results of otological (including oto-neurological) and audiological examinations With close oto-audiological cooperation a meaningful management of these cases seems possible When consistent information is revealed indicating cochlear pathology x ray of the inner meatus and complete neurological examination as a rule is not made on this basis because of the low index of suspicion towards retrocochlear findings In the group with a higher index of suspicion in this contex—comprising the minor part of the patient material—the whole clinical test battery is administered. This seems to be a justifiable and correct clinical policy probably also corresponding to Hitselberger's & House's view points

An audiologically diagnosed case of pontile angle tumor originating from N glossopharyngeus

Case 12 03 29 Male (Police officer) History hearing loss on the right ear for 1–2 years with deterioration during the last few months No tinnitus Dizziness and unsteadiness ca 1 year

Objective symptoms (Pure tone A Speech A Bekesy A Tone decay test see Fig 1) Stapedius reflex test (using a somewhat modified procedure with Madsen ZO-61 impedance meter) Stimulation of the right ear no registration on the left ear Stimulation of the left ear response at normal threshold Vestibular evaluation (*a*) no spontaneous or positional nystagmus registered by electronystagmography (*b*) cupulogram nystagmus preponderance to the left side (*c*) reduced caloric response on the right ear (30 C and 44°C) Roentgen of inner meatus destruction of apex pyramidalis (right ear)

The operation (Neurosurgical Clinic Sodersjukhuset) revealed a plum sized pontile angle tumour originating from the right glossopharyngeal nerve mostly situated within an excavated fossa in the temporal apex The facial nerve was stretched and both portions of the VIII nerve highly compressed

According to Nager (1969) a solitary pontile angle tumour originating from the glossopharyngeal nerve is extremely rare This is also substantiated by Henschen (personal communication 1971)

ZUSAMMENFASSUNG

1949 konnte Olivecrona feststellen dass weiter Fortschritte in der Handhabung der Ponswinkel Tumore nur dann zu erwarten waren wenn die Fähigkeit der Nicht Neurochirurgen die frühen Tumore zu entdecken verbessert werden konnte Dadurch war den Otologen eine klinisch Forschungsaufgabe gestellt worden Die klinische Arbeit des Verfassers hat gezeigt dass es aus otologischem Gesichtspunkt möglich war das totale Symptombild der Tumorfälle zu betrachten und sich nicht nur auf otoneurologische Konsultationen zu beschränken Spezielle topisch diagnostische Gehörsprüfungen zu entwickeln war der nächste Schritt. Als weiterere wesentliche Schritte wurden otomikroskopische Operationsmethoden entwickelt die den inneren Meatus und Ponswinkel zugänglich machten Otologie und Neurochirurgie haben damit die Entwicklung der Handhabung von Akusticustumoren karakterisiert und den Otologen immer mehr Verantwortung gegeben Der nächste Schritt in der Entwicklung ist von der Otologie und der Audiologie zu erwarten nämlich die Schaffung diagnostischer Methoden die die frühen Tumore im inneren Gehörgang entdecken konnen und die e dadurch leichter zu exstirpieren sind

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CSF SPECIFIC PROTEINS IN PERILYMPH

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(Received May 24 1971)

Abstract Using antisera against CSF and absorbed with normal human plasma two precipitate arcs formed between anti CSF well and CSF well and perilymph in two cases of acoustic neuroma This proves without doubt that the perilymph proteins have at least in part their origin in the CSF space

The origin of the protein components in human perilymph is still a controversial sub ject The capillaries in the perilymphatic space might act (e g Schreiner 1966 Silverstein 1971) as a molecular sieve allowing low mole cular weight proteins to pass easily while the high molecular weight proteins filtrate with difficulty On the other hand the proteins may derive from the cerebrospinal fluid (CSF) space via the cochlear aqueduct (Palva & Dammert 1969 Palva 1970) the loose tex tured arachnoid in the canal allowing passage of even red cells from the CSF space to the scala tympani Palva & Raunio also suggested (1967 *a*) that there is a third pathway for specific protein components into the peri lymphatic space viz through the perineural channels of the acoustic nerve

Electrophoretic analyses of both live and post mortem human perilymph have been partly unsatisfactory because of contamination by hemoglobin and serum Thus many of the high molecular weight protein bands in parti cular demonstrated for instance by Chevance et al (1960) and in many single runs by our selves (Palva & Raunio 1967 *a* *b*) were due to serum contamination Some of the runs nearly devoid of contamination showed a pre dominance of the fast moving protein bands Indeed Beck & Holz (1965) stated that un contaminated perilymph shows only a few low molecular weight protein bands

It should not be forgotten however that most of the proteins in CSF are immunologi cally the same as those in serum and originate from the capillaries Thus many perilymph proteins may actually come from the CSF even if they are identical with serum pro- teins Albumin is a case in point in the im muno diffusion tests no difference can be shown to exist between the CSF and serum al bumins but the electrophoretic mobility of CSF albumin is slightly faster than that of serum albumin Perilymph albumin has the same mobility as CSF albumin and we (Palva & Raunio 1969) therefore assumed this to be one proof that perilymph albumin actually derives from the CSF space The presence of several weak prealbumin bands in post mortem perilymph and in arachnoid acoustic nerve and brain tissue homogenate (Palva & Rau nio 1967 *a* Palva et al 1971) is in favor of the perineural pathway along the acoustic nerve This was also supported with esterase studies cholinesterases dominated in serum whereas acetylcholinesterases dominated in inner ear fluids brain tissue acoustic nerve and acoustic tumour homogenate (Palva et al 1971)

Silverstein & Schuknecht (1966) found that patients with acoustic neurinomas had high concentrations of protein in the rilym

fluid Recently Silverstein (1971) stated that this represented an accumulation of serum proteins in the perilymph However in his immunoelectrophoretic and immunodiffusion studies no antiserum against CSF was used and he thus only presented evidence for the immunological similarity of the perilymph and serum proteins As pointed out earlier because most CSF proteins are identical with serum proteins these proteins could equally well originate from the CSF space

Using antisera against CSF and absorbed with normal human plasma immunodiffusion tests were deemed necessary to get definite information on the possible specific CSF components in human inner ear fluids

METHODS

Antiserum

A potent rabbit antiserum against CSF was prepared as follows several samples of normal CSF were pooled and the pool was concentrated by ultrafiltration until its volume was one tenth of the original 2 ml of this concentrate was mixed with an equal volume of Freund s complete adjuvant and a stable emulsion was prepared White rabbits were injected intradermally each at 5 sites with 0 2 ml emulsion Immunization was carried out at 4 week intervals and 4 to 5 boosters given the same dose each time The rabbits were bled through the central artery of the ear 5 days after each booster dose Serum from each bleeding was tested by immunoelectrophoresis and the effectiveness of immunization was estimated by the number and sharpness of the precipitate lines

Absorption

To remove antibodies against serum proteins antisera were absorbed for 40 hrs at 4 C with lyophilized normal human serum (60 mg dry weight per ml rabbit antiserum) Completeness of absorption was checked by performing an immunodiffusion test on the absorbed anti CSF antisera against normal human serum

Immunodiffusion test

A sensitive microdiffusion method (Wadsworth 1957) was used modified as described earlier (Krause & Raunio 1967) The reactants were as follows concentrated CSF pool perilymph from 2 patients with acoustic neuroma and 1 patient with Meniere s disease serum from the same patients and an absorbed anti CSF antiserum The wells were charged with *10 μl* of each reactant The immunodiffusion patterns were observed daily for 3 days

RESULTS AND DISCUSSION

The 2 patients with acoustic neuroma had a high total protein (about 2 000 mg%) the case labyrinthectomized for Meniere s disease had a low protein value 300 mg% In this last case (with 10 μl perilymph) no precipitate lines were seen On the other hand both tumour perilymphs showed two precipitation arcs common to perilymph and CSF

Two precipitates which formed between the

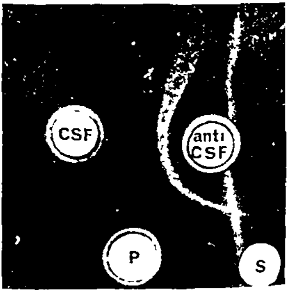


Fig 1 Immunodiffusion test in a case of acoustic neuroma Two precipitation arcs partially superimposed are seen between anti CSF and CSF and perilymph wells *CSF* = cerebrospinal fluid concentrate *anti CSF* = rabbit anti CSF absorbed by NHS *P* = perilymph *S* = serum from the same patient

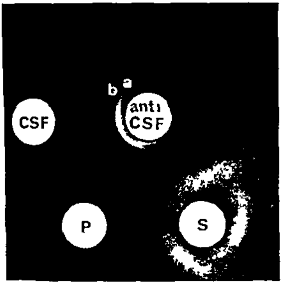


Fig 2 Immunodiffusion test in another case of acoustic neuroma Two separate precipitation arcs are seen (*a* and *b*) The weaker line (*b*) is scarcely visible between anti-CSF and perilymph wells in the reproduction The symbols are the same as in Fig 1

anti CSF well and CSF well were continuous with two superimposed lines between anti CSF well and perilymph well There were no precipitate arcs between anti-CSF well and normal human serum well showing the completeness of absorption (Fig 1) In the other tumour case the arcs were better separated one being much weaker than the other (Fig 2)

The low total protein level in Meniere's disease is apparently the reason for the absence of precipitate arcs and the high level associated with acoustic neuromas suffices for bringing out the lines in this method The result shows that at least two CSF protein constituents are present in acoustic neuroma perilymph but not in normal serum (at concentration detectable by the method used) Considering that as a rule the orifice of the cochlear aqueduct is not closed by the tumour the aqueduct seems a likely pathway for proteins

In addition to the similarities listed in the introduction between perilymph and CSF this new finding is one further definite proof that at least part of the perilymph proteins originate from the CSF space

ZUSAMMENFASSUNG

Zwei Präzipitate wurden zwischen Zerebrospinalflüssigkeit und Perilymphe und Antiserum gegen Zerebrospinalflüssigkeit absorbiert mit menschlichem Plasma in zwei Fälle von Akustikusneurinom demonstriert. Diese Erfindung zeigt ohne Zweifel dass Perilymphproteine ihren Ursprung wenigsten zum Teil in der Zerebrospinalflüssigkeit haben

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VISUAL CUE ALTERATION AND BODILY EQUILIBRIUM IN SQUIRREL MONKEYS

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(Received April 13 1971)

Abstract Bodily equilibrium maintenance ability in squirrel monkeys was behaviorally (squirrel monkey rail test) examined before and after the administration of cyclopentolate hydrochloride and homatropine eye drops The maximum ability of dynamic equilibrium maintenance declined significantly (statistically) after the administration of cyclopentolate hydrochloride eye drops however homatropine eye drops failed to produce any significant change in performance In the squirrel monkey the temporary and partial alteration of the visual cue will affect his equilibrium performance when a physically advanced task is required

The importance of the visual cues for the maintenance of bodily equilibrium has long been known both clinically and experimentally (Begbie 1966 Dickinson & Leonard 1967 Dickinson 1968) In this regard Edwards (1946) using an ataxiameter reported that experimentally blinded normal subjects demonstrated significant increase of body sway Increase of sway was found when vision was eliminated by closing the eyes or by decreasing the illumination to the point of complete darkness Reduced interference with vision was accompanied by diminution of body sway (Witkin & Wapner 1950 Wapner & Witkin 1950)

The purpose of the present paper is to report the results of the effect of temporary and partly altered visual cues as a dynamic stage *of equilibrium function in normal subhuman* primates that were trained to carry out a psycho physically advanced task

The present investigation is supported by the National Institutes of Health grants NS07237 and 5 K03 NS38619

PROCEDURE

Young adult (1 ½–2 years old) squirrel monkeys (*Saimiri sciureus*) were behaviorally conditioned to traverse the rotating rail (along its longitudinal axis) in order to measure their maximum ability to perform physically advanced locomotion The details of this procedure were previously described (Igarashi 1968 Igarashi et al 1970) The quantitation of equilibrium maintenance ability is feasible by utilizing the criterion of four consecutive falls at a certain speed of rail rotation In addition to the rail threshold values the performance profiles and points are recorded Performance points are calculated based upon the number of falls/total number of trials and the rail threshold (average of all cases at each testing time)

Upon obtaining sufficient and stable pre procedural data the subjects were restrained in the supine position seven subjects were given 1 gt of 5% homatropine (mydriatic) in each eye and 14 others were given 1 gt of 1% cyclopentolate hydrochloride (cycloplegic) in each eye The post procedural testings were

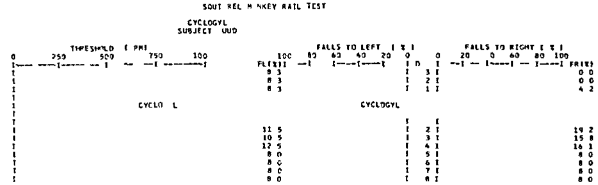


Fig 1 A computer print out demonstrates the equilibrium status after cyclopentolate hydrochloride (cyclogyl) eye drop administration Left half shows the condition of rail test thresholds (*asterisks*) In right column, the number of asterisks indicates the percentage of falls (number of falls/total number of runs on that day) and its direction Post procedural sequential numbers represent *1* 15 min *2* 30 min *3* 60 min *4* 90 min *5* 120 min *6* 150 min *7* 180 min and *8* 210 minutes after eye drop administration Note the rail threshold change and the increase of falls in this subject

performed every 30 min (in two instances beginning 15 min after the application of the medication) to avoid the fatigue effect The test was repeated until 3 1/ hours had elapsed after the administration of the medication The data analysis was performed with the aid of a computer system

The *t* test was used to detect if there was any significant change at each post procedural point (average of all subjects) when compared to the pre procedural points Rail threshold values and performance profiles were printed out

RESULTS AND DISCUSSION

Homatropine eye drops failed to produce any significant change in squirrel monkey performance on the rail Only one subject demonstrated slight decline in his rail threshold however it recovered quickly

Eight subjects out of 14 who had cyclopentolate in their eyes showed a significant change (15–60 min after the application) in rail performances (Figs 1 2) Two subjects showed slight change The other 4 failed to exhibit any change in their rail thresholds how

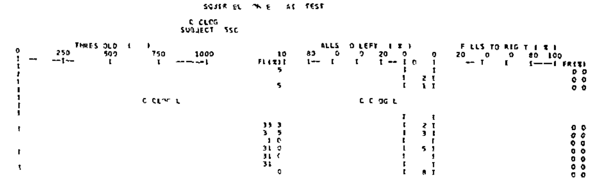


Fig 2 This print-out exhibits the rail test threshold decline (and its recovery) and the increase of percentage of falls from another subject after cyclopentolate hydrochloride eye drop administration Notice that the percentage of falls increased toward the left only and failed to decrease for a long time though the rail threshold recovered much

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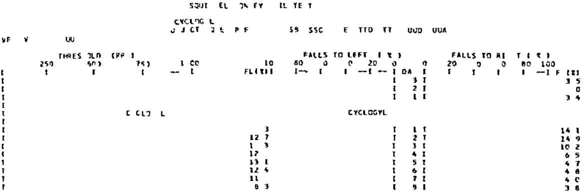


Fig 5 This computer print-out shows the average status of 14 subjects after cyclopentolate hydrochloride eye drop administration Note the changes both in rail threshold and in percentage of falls

of physical fatigue could be eliminated No subject showed any performance decline (physical) after more than 7 series of threshold measurement trials

It is known that optic stimuli increase the importance when the peripheral labyrinthine function is lost According to de Haan (1957 1959) to the extent to which the importance of peripheral vestibular perception is reduced to that extent does the significance of optic reflexes increase Further study of visual cue alteration is being planned together with partial ablation of vestibular system

Table I *Visual alteration with cyclopentolate*

The table demonstrates the result of the *t* test which was performed based upon performance points after cyclopentolat hydrochloride eye drop administration ($N = 14$) Note that significance in change was found until 60 min after the administration

	Mean	S D	*t*
Pre treatment	80 18	5 44	
Post treatment			
30 min	68 14	9 96	3 82
60 min	69 62	9 13	3 54
90 min	78 48	4 72	0 83
120 min	77 72	6 86	0 98
150 min	78 68	5 45	0 67
180 min	79 37	5 69	0 36
210 min	80 17	5 48	0 01

Significance beyond 0 005 level of probability Other *t* values were not significant at 0 05 level

ZUSAMMENFASSUNG

Totenkopfaffen (Saimiri sciureus) wurden auf die Fähigkeit ihrer körperlichen Gleichgewichtshaltung untersucht (Schienenversuch mit Totenkopfaffen) und zwar durch ihr Betragen vor und nach dem Eintropfen von zyklopentolaten hydrochloriden und homatropinen Augentropfen Der Verlust der grossten Fähigkeit ihrer dynamischen Gleichgewichtshaltung war nach dem Eintropfen von zyklopentolaten hydrochloriden Augentropfen bedeutend (statitisch) homatropine Augentropfen hatten jedoch keine bedeutende Veranderung im Betragen bewirkt Die vorubergehende und teilweise Veranderung der Sehscharfe des Totenkopfaffens beeinflußt seine Gleichgewichtshaltung wenn eine großere korperliche Leistung von ihm verlangt wird

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A MODEL DESCRIBING VESTIBULAR DETECTION OF BODY SWAY MOTION

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Abstract An experimental technique was developed which facilitated the formulation of a quantitative model describing vestibular detection of body sway motion in a postural response mode All cues except vestibular ones, which gave a subject an indication that he was beginning to sway were eliminated using a specially designed 2 degree-of freedom platform body sway was then induced and resulting compensatory responses at the ankle joints measured Hybrid simulation compared the experimental results with models of the semicircular canals and utricular otolith receptors Dynamic characteristics of the resulting canal model compared closely with characteristics of models which describe eye movement and subjective responses to body rotational motions The average threshold level in the postural response mode however was considerably lower 0 05 /sec² (S D 0 02 /sec²) Analysis indicated that the otoliths probably play no role in the initial detection of body sway motion

The vestibular system is recognized as one of the prime motion sensors involved in the control of posture In recent years extensive investigation of the physiology and input-output response characteristics of this sensor have yielded some reliable functional descriptions for reception of angular motion by the semicircular canals and of linear motion probably by the utricular otoliths In seeking to develop a model which describes how vestibular receptors contribute to postural stability however several additional details are necessary These include 1) an accurate determination of the threshold for detection of pitch and roll axis motion in the postural rather than an ocular or subjective response mode 2) a description of the relative functions of angular and linear motion receptors in the detection of body sway motion Toward answers to these questions the report proceeds with the following plan A brief review of the current literature more fully explains the need for an accurate determination of response thresholds in the postural response mode and for a quantitative delineation of the functions of the linear and of the angular acceleration sensors in control of posture Results of an experiment are presented which allow determination of the vestibular detection of body sway motion using ankle joint muscle responses as the measure of vestibular output

The experimental technique enabled elimination of all sensory cues relevent to orientation except those from the vestibular sensors Sensory cues arising from deflection of the ankle joints and changes in muscle length and tension induced by ankle joint deflection were removed by rotating the base of a specially designed platform to follow the sway motions of the body and thus maintaining a fixed ankle joint angle while allowing only isometric changes in ankle joint tension for control of posture Freefall sway was induced by translating the platform in either a forward or backward direction The delay between the initia

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tion of swaying and the subject s response to it as indicated by the onset of a compensatory muscle response at the ankle joints was measured as a function of the rate of the induced sway motion

Analysis of the experimental results provided a quantitative description of the relative contributions of the angular and the linear acceleration sensor to the detection of body sway Mathematical and computer modeling techniques were employed which consider the dynamic characteristics of the body the stimulus effect of body sway motions on each sensor and the dynamic response characteristics of each sensor The results suggested that the semicircular canals alone are responsible for the initial detection of sway motion and that function of the utricular otolith must be limited to a static and very low frequency vertical reference because of conflicting stimuli from the interaction of gravitational and body sway acceleration The threshold sensitivity of the semicircular canal response measured in a postural response mode is considerably greater than when measuring eye movements or subjective responses although the frequency response characteristics appear to be very similar in all modes

The vestibular sensors

A number of investigators observing subjective and ocular responses to semicircular canal stimulation have described relatively similar dynamic response characteristics (Van Egmond et al 1949 Groen 1956 Jones et al 1964 Meiry 1966) Experimental data however show a large uncertainty in the measured values of the angular acceleration thresholds showing a heavy dependency on the input axis the measured response modality and other physiological variables Observing subjective responses the following threshold levels have been reported Groen & Jongkees (1948) 0 28 to 2 0 /sec Hilding (1953) 0 25 to 3 0 /sec Meiry (1966) an average of 0 50 / sec Clark & Stewart (1969) an average of 0 67°/sec with values ranging from 0 06 to 2 24°/sec' Observing an oculogyral illusion to rotational stimuli the following levels have been reported Clark & Stewart (1968) 0 04 to 0 28°/sec Oosterveld (1970), as low as 0 036°/sec with significant intersubject variability It would appear that while preserving the basic frequency content of semicircular canal receptor responses transmission properties of the nervous system largely determine the sensitivity of a receptor as observed via a given response modality An accurate definition of both the dynamic response and threshold detection characteristics of the vestibular receptors in a postural response mode is therefore of critical importance to an accurate description of vestibular participation in the control of posture

The two degrees of freedom experimental platform

The experiment sought to define vestibular detection characteristics to forward and backward body sway motion about the ankle joints in the postural response mode Detection of forward and backward sway about the ankle joint was chosen because the inherently unstable inverted pendulum characteristics of the body make this the most critical mode in the regulation of quiet standing posture Motion of other body joints is of considerably less consequence during quiet standing and was neglected Hence the experimental goal was to eliminate all cues except vestibular ones which give a subject an indication that he is beginning to sway then to induce body sway and observe the compensatory response at the ankle joints

A platform was constructed which provided the base upon which the subject stood during experiments A force plate supported at each of its four corners by a miniature variable resistance force transducer (Clark Electronics Micro-ducer CS 5 100L) was used to measure ankle reaction torques transmitted to the platform base Body sway angle with respect to a vertical reference was recorded using a simple 2 potentiometer and cable system one

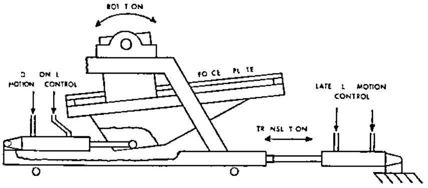


Fig. 1. A sketch of the two-degree-of freedom platform.

mounted on each hip to remove effects of vertical axis rotations of the body.

Hydraulic ram and servo valve control of the orientation of the base enabled the experimenter to independently control the deflection angle of a subject's ankle joints. A second hydraulic ram and valve system introduced forward and backward translation of the ankle joint permitting control by the experimenter of the subject's body sway angle. A sketch of the platform is shown in Fig. 1.

Measurement resolution of the force plate is ±0.10 ft lb of ankle reaction torque at frequencies to above 1,000 Hz. Linearity of the system was ±2.5° of full scale and hysteresis was within ±5° of the maximum amplitude of a cycle. The body sway angle was measured to a sensitivity of ±0.01 degrees.

Frequency response of the platform angle drive (b low ±2 degree amplitude) was flat to 5 Hz. Lateral translation velocity of the base was controlled (open loop) by a needle flow valve adjustment. Deflection velocity was adjustable between 0 and 5 inches per second equivalent to a body angle sway rate of 0 to about 7 degrees per second. Response time to maximum velocity was 10 mseconds.

Each subject stood on the platform with arms folded at the waist. He was asked to remain relaxed but to avoid conscious movements of his upper body parts and shifts in stance. Sensory feedback cues arising from ankle joint deflection were removed by rotating the platform base following the sway motions of the body and thus maintaining a fixed ankle joint angle. The effect of visual cues was determined by testing each subject with eyes opened and closed. Nulling the ankle joint angle during quiet standing effectively opened the reflex position feedback loops. Removal of reflex feedback and ankle joint deflection also eliminated any advanced cues from joint and deep pressure receptors. Platform rotation to null ankle joint angle did not interfere with the subject's ability to generate necessary isometric ankle torques to maintain postural stability.

Body sway was induced using very small velocity forward and backward platform displacements. These displacements were induced only after the ankle torque and sway angle measurement had remained constant for at least one second, assuring that the observed response was the result of the induced body motion only. A hybrid computer recorded ankle reaction torque and body sway angle data on digital tape during each response sample. A subject was asked to remain standing for only 16 such samples at a time (about 3 min) to avoid fatigue. Ankle torque responses to induced sway rates ranging from 0.15 to 3.0 deg/sec were observed. Complete test sequences with eyes opened and with eyes closed were conducted on three healthy normal subjects aged 19, 21 and 26.

RESULTS

Typical responses to a rapid or to a slow induced body sway stimulus were cha

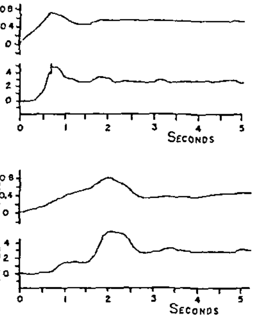


Fig 2 Typical responses to induced sway motion 1 1 degrees sec in the upper record and 0 2 degrees/sec in the lower record Traces show body sway angle in degrees and ankle reaction torque in foot pounds

by an initial period during which the body angle began to increase while the ankle reaction torque remained constant (Fig 2) Then motion was detected by the vestibular receptors and the ankle reaction torque increased rapidly returning the body to a stable orientation

The threshold for detection of body sway was defined as the time from initiation of platform motion to the time at which the ankle torque level had increased by 0 25 ft lb above its initial level Because all subjects tended to oscillate continuously with their eyes closed and ankle joint angle fixed an accurate vestibular threshold function could not be determined for this case Instead the nulling procedure above was modified so that when subjects were tested with eyes closed the platform rotated to null only the induced component of body angle sway To show that this altered procedure did not introduce significant reflex and kinesthetic cues a similar test was made with eyes open and the results compared to those in which all ankle joint angle motion was nulled

The threshold characteristics (Fig 3) showed detection time delay as a function of the sway rate induced by the platform Variations among the three subjects and among the three test conditions were statistically insignificant ($p > 0.1$)

A consistent relationship between postural response time and initial free fall sway rate of the body was found Since the results of all three test procedures were the same it was concluded that only vestibular feedback contributed to the observed detection characteristics However the raw data did not indicate relative participation of the linear and angular acceleration receptors Therefore to provide a reasonable description of the relative contributions of these receptors to body sway detection analytical and computer modeling techniques were employed The body dynamic characteristics the stimulus effect of body motion on each sensor and the dynamic response characteristics of each sensor were considered

Body sway stimulus effect on the vestibular receptors

Angular acceleration of a rigid human body about the ankle joints is directly equivalent to the accelerating input to the pitch axis semicircular canals Since the vestibular models to

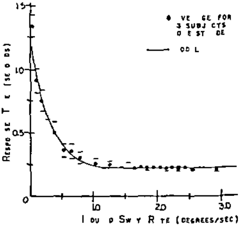


Fig 3 Postural response delay time as a function of induced sway rate the experimental data and simulation of the pitch axis semicircular canal model

be developed consider only quiet standing during which time the upper body remains relatively rigid equivalence of body sway acceleration and pitch axis angular acceleration was assumed

The effect on linear motion receptors from body sway is considerably more complex since both gravitational and linear acceleration reaction forces act as inputs During free fall sway of the body gravitational and tangential acceleration reaction forces act in opposite directions as shown by equations 1–3

The body dynamic equation is

$$I_B \ddot{\theta}_B = m_B g h_g \theta_B + T_A \tag{1}$$

The net reaction force on the linear motion receptor is

$$f_0 = g\theta_B - d_0 \ddot{\theta}_B \tag{2}$$

During free fall ($T_A = 0$) the net reaction force is

$$f_0 = \theta_B \left[g - \frac{m_B g h_g d_0}{I_B} \right] \tag{3}$$

Where

$\theta_B = \sin\theta_B$ small angle approximation for the body sway angle
I_B = moment of inertia of body about the ankle joints
h_g = height of the center of mass
m_B = mass of the body
d_0 = height of the receptor above the ankle joints
T_A = reaction torque about the ankle joints

Because the utricular otolith receptors are located well above the center of mass in the human the net reaction force on these receptors is negative during an unrestrained fall (i e it is opposite to the force on the receptor due to gravity alone) If the body sways with partial resistance from postural muscles ($T_A > 0$) the net force on the linear motion receptors may be negative zero or positive depending on the intensity of the resistance

Assuming that body sway is restrained with an ankle torque roughly proportional to angular displacement of the ankle joint the following equations demonstrate the ambiguity of the sign of the linear motion stimulus

Let $T_A = K\theta_B$

$$f_0 = \theta_B \left[g - \frac{m_B g h_g d_0}{I_B} + \frac{K d_0}{I_B} \right] \tag{4}$$

Using average body parameter values (Dempster 1967) we find that for swaying forward

sign $f_0 > 0$ if $K > 4$ ft lb/degree
sign $f_0 < 0$ if $K < 4$ ft lb/degree

The value of gain (K) necessary for static stability of the body to sway is approximately 7 ft lb/degree

The vestibular sway detection models

The experiment induced postural sway by displacing the platform in either a forward or backward direction at constant velocity The following equations describe the effect of these lateral displacements on the body sway motions and motion receptors

The lateral displacement velocity (V_A) introduces an angular acceleration impulse to the body

$$\ddot{\theta}_B = U_0(t) \left[\frac{V_A}{h_g} \left(1 - \frac{M_{EQ}}{m_B} \right) \right] \tag{5}$$

where $U_0(t) = 1 \quad t = 0$
$\qquad\qquad = 0 \quad t \neq 0$

$$M_{EQ} = M_B + \frac{I_g}{h_g}$$

I_{cg} = body moment of inertia about the center of mass

The displacement also introduces a lateral acceleration impulse to the head

$$\ddot{Y}_0 = U_0(t)(V_A) \left[1 - \frac{d_0}{h_g} \left(1 - \frac{M_{EQ}}{m_B} \right) \right] \tag{6}$$

The impulses act to set the initial conditions of the body sway and receptor dynamic equations (1) and (2) So

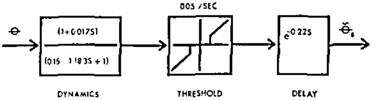


Fig 4 The pitch axis semicircular canal model for detection of body sway motion The input is body sway acceleration The output is an estimate of body sway rate S is the LaPlace transform operator

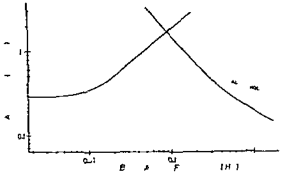
the platform continues at constant velocity no further lateral forces act between the body and the platform and equations (1) and (2) completely describe the inputs to the angular and linear motion receptors

Second order dynamic models of the angular and linear motion receptors with threshold and time delay were simulated using a PDP8 GPS hybrid computer facility The model for the pitch axis semicircular canals which predicts the experimentally observed detection characteristics over the entire range of induced sway rates is presented in Fig 4 The detection characteristics of the semicircular canal model are included in Fig 3 for comparison with the experimental data

Simulation of an otolith model in the form presented by Young & Meiry (1968) indicated that the linear acceleration threshold values must be an order of magnitude less than the lowest values reported in the literature even in the limiting case as the induced sway rate approaches zero The linear motion sensor model is sufficient to account for observed response threshold levels only in the static or nearly static range since the body free fall rate ($W_B \approx 3$ rad/sec) is fast compared to the very slow dynamics observed for these receptors In view of this result and the finding that otolith response to postural sway motion may be ambiguous it was concluded that the linear motion receptors probably play no role in the detection of postural sway

Dynamic characteristics of the pitch axis semicircular canal model which predict the observed sway detection characteristics compare very closely with those of Meiry s (1966) model The addition of a very small lead term is necessary to predict the minimum response time characteristics for large impulse inputs and has no appreciable effect on response properties within the frequency range of normal body motions The model defined a vestibular angular acceleration threshold value of 0 05°/sec with a standard deviation of approximately 0 02°/sec for the pitch axis canal in the postural response mode Total response time delay, including delay within the sensor processing within the brain transmission to the postural muscle and the generation of muscle tension is 225 msec

The average threshold value for the pitch axis canals in the postural response mode is significantly lower than any observed with subjective or oculogyral illusion responses The threshold values also show consistency from trial to trial and subject to subject Functional, neuroanatomical and performance criteria seem to confirm these experimental findings All methods of measuring vestibular responses in normal human subjects necessarily introduce characteristics of additional neural and muscular elements between the actual detection of motion by the receptor and the observed response to the motion Characteristics of these additional elements are unique for each response modality and are also subject to modification by other processes within the central nervous system Pitch axis motion feedback is critical in the control of human posture Hence functional considerations confirm the highly sensitive and consistent link observed between pitch axis motion detection by the vestibular system and the appropriate stabilizing postural muscles Neuroanatomical studies of vestibular projections to postural muscles have demonstrated strong functional links For example microelectrode stimulation



Fi 5 The amplitude of a sinusoidal body sway just large enough to be detected by simulated models of the vestibular linear and angular motion receptors Th otolith model is from Young & Meiry The canal model is shown in Fi 4

of the lateral vestibular nucleus of the cat produced excitatory postsynaptic potentials in extensor muscles of the hind limb in the cat *while flexors were inhibited (Lund & Pom* peiano 1965) Their excitation effects were shown to be monosynaptic Observations of Yemelyanov et al (1967) show how the task at hand may alter the characteristics of the *neural interface between a vestibular input* and a given measured response They observe that while balancing himself a man s vestibular sensitivity thresholds decrease two or three fold

An explanation as to why only the semi circular canals contribute to the detection of body sway is found by observing the body sway frequency ranges at which each sensor is most effective in detecting the motion (i e its *detection threshold is lowest) Fig 5 shows the* sinusoidal body angle amplitude necessary to just achieve detection of motion as a func tion of sway frequency Note the clear separa tion of the operating range for each sensor *Together the linear and angular motion re* ceptors provide sensative detection of sway motion over the entire range of normal body sway motions

In summary several significant points rele *vant to properties of* the vestibular receptors and their role in the control of posture have been presented

1 Pitch axis semicircular canal dynamic response characteristics measured in the pos tural response mode compare closely with those found using eye movement and subjec tive response data

2 The average threshold level for the pitch axis canals in the postural response mode was found to be 0 05 /sec with a standard devia tion of approximately 0 02°/sec Variations in this value among the three subjects tested were statistically insignificant

3 A consideration of the stimulus effects of body sway motion on the pitch axis canals and utricular otoliths strongly suggested that *the otoliths play no part in the detection of* body sway motion Otolith response must be limited to very low frequencies to eliminate a possible sign ambiguity in its response Dur ing posture control the primary function of *the otolith is most likely as a static vertical* reference

ZUSAMMENFASSUNG

Eine experimentelle Technik wurde entwickelt wel he die Formulierung eines quantitativen Modells mo lich machte das die Vorhofentdeckung von Korp rschwan kungen in einer positionellen Reaktionsweise be schreibt Alle Fingerzeige — au er denen des Vor hofs — welche einer Versuchsperson einen Hinweis gaben daß sie zu schwanken begann wurden mittels einer speziell konstruierten in zwei Richtungen be weglichen Plattform eliminiert dann wurden Kor perschwankungen herbeigefuhrt und die sich erge benden ausgleichenden Reaktionen an den Fuß knoch In gemessen Mischsimulationen verglichen die experimentellen Ergebnisse mit Modellen der halb*kreisformigen Gange und der utrikularen Gehorstein* chen Dynamische Charakteristiken des sich erge benden Gehorgangmodell kamen denen jener Mo delle sehr nahe welche die Augenbewegung und sub jektiven Reaktionen zu Rotationsbewegungen des Kor pers beschreiben Der durchschnittliche Grenzwert in der po itionellen Reaktionsweise war jedoch erheblich niedriger 0 05 °ec' (S D 0 02 sec°) — Die Analyse ergab Anzeichen, daß die Gehorsteinchen wahr scheinlich keine Rolle in der anfän lichen Entdeckung korperlicher Schwankungen spielen

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THE IMPORTANCE OF SIALOGRAPHY FOR THE DETERMINATION OF THE PAROTID FLOW

The Normal Variation in Salivary Output in Relation to the Size of the Gland at Stimulation with Citric Acid

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Abstract The relation between the size of the parotid gland and the output of saliva has b en studied in 9? healthy individuals of both sexes and of various ages The size of the gland has been determined on sialograms and the relation between the gland size and th secretion stimulated with different citric acid solutions has been studied with regression analysis A formula for the predicted secretion with varying siz s of the parotid gland has been set up and the relation has been graphically illu trated Th study has shown that the secretory rate of the parotid gland should b related to the size of the gland when there is a matter of assessing the function of th parotid glands and that a sialographic investigation of the parotid may be taken as a basis for a prediction of the no mal secretion range of a gland The clinical value of the sialometric method as a diagnostic aid in the investigation of diseases of th salivary gland has in this way been considerably increased

Recording of the flow of saliva (sialometry) can be used as a diagnostic tool for the discovery of pathological changes in the salivary glands as such changes commonly lead to an altered function in the glands (Diamant 1960 Enfors 1962 Sewards et al 1966 Ericson & Jacobsson 1968) However owing to the fact that the secretion of saliva normally shows great individual variations it is as yet not possible in the individual case to use the method with a reasonable hope of accuracy where it is a matter of differentiating between healthy glands and glands with moderate or small disturbances in the function With the increased knowledge of which factors are correlated to the secretion of saliva and the factors contribution to the variance in the secretion it is possible with a greater margin of safety than with the traditional comparisons of mean values to decide whether the function in a certain gland is normal reduced or intensified

In order to make a closer study of the background to the normally great individual differences in the rate of salivary secretion in healthy persons Ericson (1968 1971) studied the effect of a large number of factors on the variability of the parotid secretion with multiple regression analysis In all forty nine different variables which theoretically or according to earlier investigations might influence the rate of the salivary flow were studied The results showed that many factors are significantly correlated to the secretion and that these factors may vary both in character and in importance as between secretions stimulated in different ways However without comparison the most important factor of all in explaining individual differences in the parotid flow rate on stimulation wi citric acid proved to be differences in th

of the parotid glands The analyses indicated that by relating the secretion to the glandular size it might be possible to improve the sialo metric method and increase its diagnostic value

The aim of the present study is to investigate the salivary secretion of the parotid gland in healthy subjects on stimulation with differ ent citric acid solutions in relation to the size of the gland

MATERIAL

The material consisted of 92 healthy adult in dividuals 61 females and 31 males chosen in a random way from the clientele of the School of Dentistry in Umeå The age of the 61 women varied between 26 and 64 years the average being 49 8 years while the age of the 31 men ranged from 26 to 64 years with an average of 48 8 years

The subjects were examined with regard to their general health No diseases or condi tions were diagnosed which in the light of earlier experience gave rise to secretory dis turbances

The parotid glands were studied clinically and roentgenologically with sialography No definite pathological conditions were diag nosed Three glands from three subjects did however show on the sialogram an appear ance differing somewhat from the normal and from the appearance of the gland on the other side but here too there was no history of pathological salivary conditions and the sub jects themselves were healthy For a detailed account of the material the reader is referred to Ericson (1968)

METHOD

Sialometric method

The method applied for the determination of the secretory rate has been described earlier by Ericson (1968 1969) A brief account of the procedure is given below

The determinations of the secretory rate were made during stimulation with 1 6 and 10% citric acid solutions During the deter minations the patient reclined on a couch with the sagittal plane of the head parallel with the vertical plane The salivary output from each gland was simultaneously collected with a sialometer placed over papilla salivaria in vestibulum oris and via a water filled system connected with a drip-chamber, where the drops from each gland were automatically re corded when falling past a photoelectric counter Each recording implied one drop of 0 06 g of saliva

The citric acid was applied at the midline of the anterior part of the dorsum of the tongue with three drops every 30 sec for 5 min when 1% citric acid solution was used as stimulus and with three drops every 15 sec for 2 min in the case of the 6% and 10% solutions The subjects were instructed to move the tongue after every application and to swallow to ensure that the solution should be distributed evenly over the tongue and so that the stimulatory effect of the citric acid should not be affected by retention of saliva Between the recordings the mouth was care fully rinsed with water and the tubes flushed out The interval between the periods of stimulation varied according to the rapidity with which the glands returned to resting secretion A fresh stimulus was administered not less than 5 min after this

Analyses of the sialometric method were carried out (Ericson 1968 1969) These show that errors due to the application of the stimu lus and to the recording apparatus are slight as compared with the variation found among the subjects and that the biological variation between the subjects is greater than the intra individual variation and the errors due to the apparatus (Ericson 1968) The correlation co efficient r between two recordings with an interval of at least 1 week was for 1 6 and 10% citric acid stimulation $r=0\ 96$ $r=0\ 95$ and $r=0\ 92$ respectively The mean value and the standard deviation for the right and left glands were of the same order of magnitude

Radiographic examination

The radiographic examination of the right and left parotid glands was performed with sialography the method described and analysed by Ericson (1968) being used in this connection The beam was directed towards the midpoint of the gland in the lateral projection at an angle of 78 degrees to the sagittal plane and in the anteroposterior projection perpendicular to the plane of film and parallel to the ramus ascendens of the mandible (Ericson 1970) The film to focus distance was 70 cm

The sialograms were taken as the basis for an estimation of the size of the glands and the quantification of the regressor which in the analysis represents the gland size The method launched by Ericson (1968) and studied by Ericson & Hedin (1970) was used for the determination of the size of the gland Methodological studies of this method showed that there is a high correlation between the extent of the parotid gland on the sialogram and *in situ* ($r=0\ 97$) as the correlation between the extension of the gland in the sagittal and frontal planes ($r=0\ 82$) The high correlation between the lateral and frontal dimension of the gland and the accuracy of which the sialography reproduces the outlining of the gland makes it possible with a high degree of accuracy to express the relative size of a gland only with knowledge of one of these parameters Studies of autopsy material have shown that the correlation between the lateral extent of the parotid gland and its actual volume also is very high $r=0\ 96$ (Ericson & Hedin 1970)

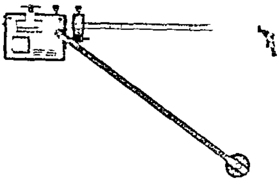


Fig 2 Aristo planimeter no 1130 used for the calculations of the glandular extension in the sialogram



Fig 1 Sialogram of parotid gland in lateral projection the outer boundary of the gland is traced.

In the regression analysis below the size of the parotid gland is denoted by the area of the lateral extension of the gland as projected on the lateral sialogram In the mathematical analysis (linear) this variable substituted the actual volume

The procedure for the determination of the size of the gland was briefly as follows The outer boundaries of the gland were traced with a pen and the enclosed area was computed with an Aristo planimeter (No 1130) in mm^2 (Figs 1 2)

For a detailed account of the method and for methodological studies the reader is referred to the works mentioned in the foregoing (Ericson 1968 1970 Ericson & Hedin 1970) The systematic errors have been small

Data processing

The aim of the mathematical analyses was to calculate the upper and lower limits for the parotid secretion in healthy persons for different glandular sizes

Table I *The mean values the errors of the mean (S E) and the standard deviation (S D) for the parotid flow on stimulation with citric acid on the right and left sides*
N = 92

Stimulus	Number of drops Mean ± S E	S D	Range
1 % citric acid R/5 min	54 0 ± 3 7	35 4	16–287
1 citric acid L/5 min	51 0 ± 3 5	33 2	7–239
1 citric acid R + L/5 min	105 0 ± 6 9	66 4	27–526
6 % citric acid L/2 min	60 0 ± 2 9	27 4	23–168
6 % citric acid R/2 min	59 5 ± 2 8	26 6	11–216
6 % citric acid R + L/2 min	119 4 ± 5 4	52 8	34–384
10 citric acid R/2 min	68 4 ± 2.7	26 2	19–211
10 citric acid L/2 min	68 5 ± 2.7	26 2	24–148
10 citric acid R + L/2 min	137 0 ± 5 3	50 6	46–354

The relation between the variation in secretion and glandular size was analysed with regression analysis A large number of preliminary regression analyses was carried out with the secretion after stimulation with 1 6 and 10 % citric acid as regressand and different transformations of the planimetric measurements of the size of the gland as regressors In this connection the original glandular surface area has been tested as measured on lateral and frontal sialograms diameters squares roots and logarithmizations

The analyses showed that the highest correlation between secretion and gland size was throughout obtained for the logarithmized secretion and the logarithmized value of the glandular extension in the lateral sialogram It is these analyses that have been taken as the basis for the final account of results

The relation between the log value of the parotid secretion (the regressands) and the log value of the size of the gland (the regressor) was analysed with a weighted regression analysis according to the equation $y_{pred\,i} = b_0 + b x_i$ where $y_{pred\,i}$ is the predicted log value of secretion b_0 is a constant and b the regression coefficient and x_i the log value of the size of the gland for an individual i The regression coefficient was calculated with the method of least squares and the weight was chosen in accordance with current rules i e inverse proportional to the square of the linear expression

The calculations have been carried out with the program WRAP (see Ericson 1968) where y_i is defined as the natural logarithm of the secretion value of both parotid glands for an individual i and x as the natural logarithm of the sum of the lateral extension of the right and left parotid glands as projected on the sialograms This program gives besides the mean values x standard deviation S D standard errors, S E x regression coefficients b total correlation coefficients R and the standard error of the individual y value for every x_i value δ^* One gets an upper and a lower limit for the normal parotid secretion by putting $\pm 2\delta^*$ to the predicted salivary secretion

RESULTS

In Table I are presented the mean value the standard error of the mean value the standard deviation and the range for the salivary secretion from the left and right parotid glands after stimulation with 1 6 and 10% citric acid and in Table II the corresponding values for the area of the parotid gland as projected in the lateral sialograms Both secretion and size of gland show good agreement between the right and left sides From Tables I and II it appears that the variation between individuals as regards both secretion and size of gland is great

Table II *The mean value the error of the mean (S E) and the standard deviation (S D) for the area of the parotid gland in the sialogram in a lateral projection*
N = 92

Side	Mean ± S E	S D	Range
Right gland in mm	1553 ± 30 1	289	990–2100
Left gland in mm²	1559 ± 30 2	290	590–2100
Right and left glands in mm²	3112 ± 56 9	546	1580–4190

Table III *Regression coefficients and intercept constant their (S E) and level of significance (marked with asterisks) correlation coefficient and the equation of the predicted secretion*

Population 92 healthy subjects

Regressand parotid flow rate (R+L) stimulated with 1% citric acid drops/5 min. Log value

parotid flow rate (R+L) stimulated with 6 citric acid drops/2 min Log value

parotid flow rate (R+L) stimulated with 10% citric acid drops/2 min Log value

Regressor X log. lateral projection of parotid glands in the sialograms in mm²

* significantly different from zero ($P<0\ 001$)

1	Intercept constant ± S E	−10 22*** ±1 33
	Regression coefficient ± S E	1 84** ±0 17
	R=0 77	
	$y_{pred}=-10\ 22+1\ 84\quad X$	
6%	Intercept constant ± S E	−6 07*** ±1 26
	Regression coefficient ± S E	2 01*** ±0 24
	R=0 70	
	$y_{pred}=-6\ 07+2\ 01\quad X$	
10	Intercept constant ± S E	−5 76*** ±0 38
	Regression coefficient ± S E.	1 98 ** ±0 08
	R=0 93	
	$y_{pred}=-5\ 76+1\ 98\quad X$	

The relation between salivary secretion and gland size has been studied with a weighted regression analysis in which the weight has been chosen in inverse proportion to the square of the linear equation expression It is on the regression analysis that the reported results have been based In Table III are given *the regression coefficients and equations for the logarithmized predicted parotid secretion* after stimulation with citric acid in different concentrations Table III also gives the correlation coefficient (R) for the variable included in the analyses The correlation coefficient (Table III) indicates the degree *in which in the equations the regressor (gland* size) contributes to explain the variance of the regressand (secretory rate) A comparison between the regressands shows that the lowest value for R is attained for the secretion stimulated with 6 % citric acid ($R=0\ 70$) and the highest value for the secretion stimulated with 10 % citric acid ($R=0\ 93$) These values are considerably higher than the values for the simple correlation coefficients obtained in the

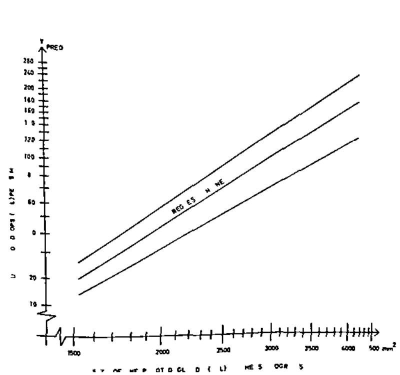


Fig 3 The relation between the lateral extension (in mm²) of the parotid glands (R+L) and th flow of saliva on stimulation with 1% citric acid solution (regression line) The upper and lower lines in th diagram represent an approximate 95% prediction interval Double logarithmic scale

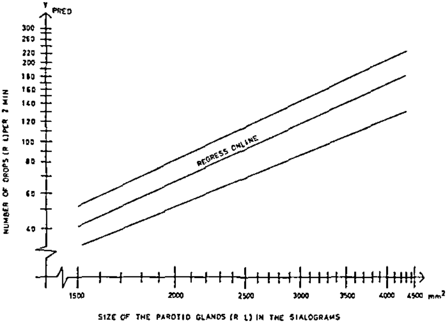


Fig 4 The relation between the lateral extension (in mm²) of the parotid glands (R + L) and the flow of saliva on stimulation with 6% citric acid solution (regression line) The upper and lower lines in the diagram represent an *approximate 95% prediction interval* Double logarithmic scale

primary unweighted analyses (compare Ericson 1968)

The relation between size of gland and secretion is shown graphically in Figs 3–5 On the y-axis is given the secretion stimulated with 1 6 and 10% citric acid and on the x axis the lateral extension of the parotid gland on the sialogram in mm The diagrams are drawn on a double logarithmic scale (y and x axes) The relation between the average secretion of saliva and the surface area of the gland is reproduced with the regression line and the deviation around the mean value according to the 2 sigma rule Thus the upper and lower lines in the diagrams enclose a prediction interval within which about 95 of the series

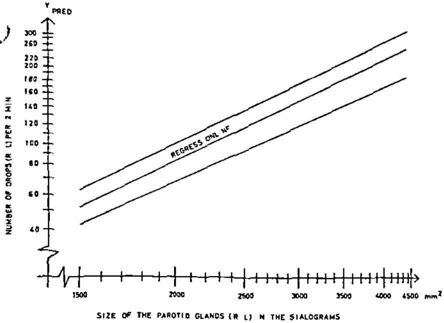


Fig 5 The relation between the lateral extension (in mm²) of the parotid glands (R + L) and the flow of saliva on stimulation with 10% citric acid solution (regression line) The upper and lower lines in the diagram represent an approximat 95 prediction interval Double logarithmic scale

lies as the residuals were approximately normally distributed For this material the secretion values of each subject have been plotted in Figs 3–5 It was shown that 97% of the values falls within the intervals As emerges from the diagram the standard deviation in secretion varies with the size of the gland and increases with increasing gland size

DISCUSSION

The choice of the subject material was determined by an earlier investigation (Ericson 1968) A certain deviation from a formally *randomized population is therefore to be expected* It has not been possible to analyse to what extent the patient material differs with respect to the parotid function from a strictly random selected material and the eventual significance of this for the results However the subjects studied came from different social classes and were all clinically healthy No state of dehydration nutrition deficiency renal or metabolic abnormality which might influence the salivary output and the validity of the results was observed Neither did I observe anxiety before the sialometric determination to an extent which possibly could have an effect upon the stimulated secretion

The results obtained show that in healthy subjects the individual variations in the size of the parotid glands explain to a very large extent the normally great individual differences in the secretory rate of the parotid gland The importance of the size of the gland for the variance in secretion is not however constant but varies somewhat between stimuli Of the greatest importance for the variance is the variable for the secretion stimulated with 10 citric acid ($R = 0\ 93$) but also for the secretions stimulated with 1 and 6 citric acid the variable gets high explanatory values ($R = 0\ 77$ and $0\ 70$ respectively) These values are higher than corresponding ones obtained for the unweighted analyses (Ericson 1968)

This is most pronounced for the secretion stimulated with 10% citric acid The strong correlation between the parotid output and the size of the parotid glands for all stimuli indicate that other factors present which might influence the normal flow rate are of subordinate importance when the size of the glands and the errors of the sialometric and sialographic methods have been taken into consideration The results show that in practice no reliable individual assessment of the function of the salivary gland should be made without relating the volume of the secretion to the size of the gland

From the diagrams it emerges that even after *taking into account the size of the gland* the variation about the regression lines is appreciable However as compared with the standard deviation for the mean value of the whole population there has been a considerable reduction in the variance after the size of the gland has been taken into account

In the analysis the lateral extension of the gland on the sialogram is used as the index for the size The reason why this expression has been taken instead of the actual volume is that the lateral extension is clinically easier to calculate exactly than is the volume and that the lateral surface area and the volume show a very high correlation They may thus replace each other in the linear analysis ($r = 0\ 95$) (Ericson & Hedin 1970)

In tables and diagrams the parotid secretion from the right and left sides have been added together as have also the surface areas of the glands projected on the lateral sialograms As the mean values and standard deviations are almost the same for the left and the right sides as regards both secretion and glandular size the predicted secretory value for one gland can be approximated only by halving the values in tables and diagrams

Analyses of the drop-size have shown that every drop corresponds to 0 06 g of saliva and that plausible variations in the rat secretion not to any extent worth men affect the size of the drops (Eri A conversion of the unit used in t

drops per time unit to grams per time unit may be made without the regression lines or intervals being changed

In Figs 3–5 is shown the relation between secretion and size of gland Besides the regression line which describes the predicted mean value for pairs of glands varying between 1 500 and 4 500 mm² the diagrams give an upper and lower limit for the variation calculated according to the two-sigma rule ($\bar{x} \pm 2$) Thus the upper and lower lines in the diagrams mark a prediction interval within which about 95% of the secretion values should fall. In view of the fact that the intervals are based on results from healthy individuals the intervals may be considered to represent the normal range of variation on stimulation with different citric acid solutions and may be used clinically as a reference frame to decide whether a certain secretion value is to be considered normal or pathological In this way it is possible to make a more reliable assessment of individual secretory response than has earlier been the case

ACKNOWLEDGMENT

The author wishes to express his thanks to Associate Professor G Eklund Ph d for statistical advice

ZUSAMMENFASSUNG

Das Verhältnis zwisch n der Grosse der Glandula Parotis und dem Speich lfluss wurde an 92 gesunden Person n v rschied nen Alters und Geschlechts untersucht. Di Grosse der Speicheldruse wurde an Sialogrammen bestimmt und di Relation zwisch n der Grösse der Druse und der mit verschiederen Zitronensaurelösungen stimulierten Sekretion mit Hilfe der Regressionsanalys studiert. Eine Formel für die zu erwartende Sekretion bei verschiedenen Grossen der Drüsen wurde aufgestellt und die Relation graphisch illustriert. Die Untersuchung hat gezeigt, dass bei der Funktionsbestimmung die Sekretionsrate zur Grösse d r Drüse in B ziehung gesetzt werden muss und dass die sialographische Untersuchung als Basis für die Grösse der zu erwartenden normalen Sekretion benutzt werden sollte Der klinische Wert d r sialometrischen Methode als diagno tisches Hilfsmittel für die Erforschung der Krankheiten der Speicheldrüs n wurde auf di e Weise betrachtli h erhöht

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PSEUDOANEURYSM OF THE INTERNAL CAROTID ARTERY

A Warning of a Septic Erosion

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(Received March 28 1971)

Abstract Swelling of the upper part of the neck and parotid region combined with swelling of the lateral wall of the pharynx after a pharyngeal infection (pharyngitis or tonsillitis) indicates a parapharyngeal abscess This abscess may be complicated by involvement of the nerves and vessels passing this region Thus in our cases neurological symptoms from n glossopharyngeus n vagus n accessorius and n hypoglossus and the cervical sympathetic nerves were seen as well as erosion of the internal carotid artery On erosion of an artery either the vessel wall is perforated directly or generally a pseudoaneurysm may form before the rupture of the vessel Therefore early diagnosis of a pseudoaneurysm of the internal carotid artery is very important so that ligation can be done before the often fatal rupture It is demonstrated that repeated minor haemorrhages from the nose and throat and Horner s syndrome are typical symptoms of a pseudoaneurysm of the internal carotid artery The reason why the sympathetic nerve is affected may be the close anatomical relationship between the internal carotid artery and the sympathetic plexus running along the artery upwards through the carotid canal The importance of early diagnosis of the pseudoaneurysm and immediate ligation of the internal carotid artery is stressed In order to avoid unnecessary ligation an angiography should be performed to verify the diagnosis The incidence of cerebral complications following ligation of the internal carotid artery is discussed

A parapharyngeal abscess usually results from pharyngeal and tonsillar infection by spread of the infection through peritonsillar veins and lymphatics but may also arise from infected lymph nodes draining other surrounding areas than the pharyngeal and tonsillar regions

A parapharyngeal abscess may be complicated by involvement of the nerves and vessels passing the parapharyngeal space and the most serious complication is a septic erosion of the internal carotid artery

Before the antibiotic epoch several cases of parapharyngeal abscesses involving the internal carotid artery were reported (Salinger & Pearlman 1933) but on account of the effectiveness of antibiotics in preventing this complication only a few reports have been found in the literature since 1945 (Weber 1950 Folken 1951 Greven 1954 Hogarth 1959 Eckel 1960 Todman 1960 Masing 1961)

Thus the signs and symptoms of a parapharyngeal abscess and its complications are nowadays very seldom observed and may therefore easily be overlooked

This is illustrated by the following cases which especially demonstrate the importance of diagnosing a pseudoaneurysm of the internal carotid artery—a warning of a septic erosion

Case 1 A boy aged 5 years The boy was admitted to the hospital after suffering for 1 week from dysphagia and a painful swelling below the angle of the left jaw A diagnosis of epidemic parotitis was primarily suggested but on admission to the hospital a peritonsillar swelling on the left side was observed and incised but no pus escaped A Horner syndrome was also observed The child was treat

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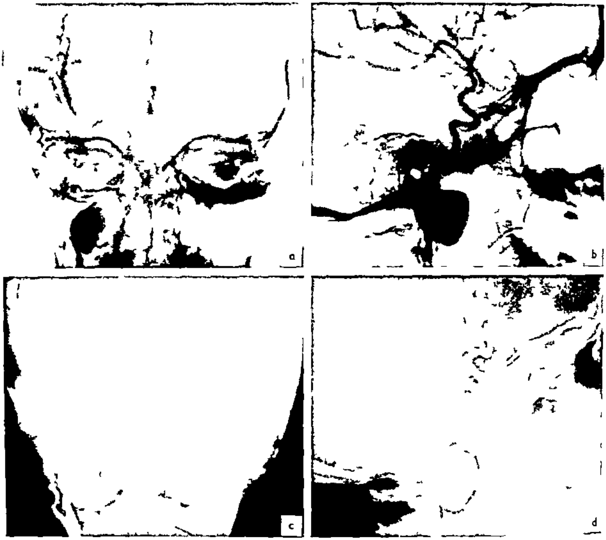


Fig 1 Pseudoaneurysm of the internal carotid artery (a) Frontal view (b) Side view (c d) Subtraction photographs

massive doses of penicillin and the swelling on the neck and in the pharynx subsided completely within the next 2 weeks A decreasing blood haemoglobin and a positive Weber were noticed As no bleeding from the nose or throat could be seen gastrointestinal bleeding was suspected Two weeks after admission to the hospital a hypoglossal nerve paralysis was observed and after another week the boy suddenly had a severe haemorrhage from the mouth and nose lasting a few minutes Carotid angiography revealed a big aneurysm on the internal carotid artery just below the base of the skull (Fig 1) In order to facilitate control of repeated bleeding the carotid artery was immediately exposed in the bifurcation region However as the nature of the aneurysm was not quite clear and the possibility of its exploration for resection and subsequent grafting was discussed no ligation was performed but cotton strips were placed around the common and internal carotid arteries However 15 minutes later a sudden severe bleeding occurred from the nasopharynx Tying the cotton around the common and internal carotid arteries did not stop the bleeding but reduced it After another

.0 minutes the bleeding gradually stopped The cotton strips were replaced by silk ligatures The boy made an uneventful recovery No sign of brain damage was found The Horner syndrome and the hypoglossal nerve paralysis disappeared gradually during an observation period of 4 months postoperatively

Case 2 A man aged 18 years Fell acutely ill with pain in the throat and fever and increasing swelling on the right side of the neck and in the region of the right parotid gland Considered to be a case of epidemic parotitis After 7 days the condition had deteriorated with trismus and increasing pain on the right side of the neck Antibiotic treatment was given An incision was made on account of suspected peritonsillitis but no pus escaped After a further 2 days the patient had severe recurrent haemorrhages from the nose and therefore visited the Department of Otolaryngology where he was hospitalized Massive doses of penicillin were given and a debridement of the incision opening was done again without escape of pus On the first 3 days after admission to hospital his condition was relatively unchanged with recurrent small haemorrhages from the nose trismus dysphagia and swelling on right side of the neck On the fourth day in hospital i e 14 days after the onset a Horner syndrome and hypoglossal nerve paralysis developed and the patient had very severe haemorrhages from the nasopharynx the source of which could not be traced Carotid angiography however revealed a large aneurysm on the right internal carotid artery which was thrombotized cranially to the aneurysm To prevent a fatal haemorrhage the internal carotid artery was ligated Apart from the aforementioned neurological symptoms a right sided paralysis of the accessory and recurrent nerves and of the pharynx was also observed as well as a diminished sensibility in the rear right hand part of the tongue The postoperative course was uneventful No sign of brain damage was found postoperatively During an observation period of 5 months postoperatively the paralysis of the recurrent nerve had not entirely disappeared while the Horner syndrome and the paralysis of the glossopharyngeal the accessory and the hypoglossal nerves gradually disappeared during this period

Diagnosis of the parapharyngeal space infection

The parapharyngeal space has the shape of a pyramid with the apex pointing to the hyoid bone and the base formed by the base of the skull The medial face is formed by the fascia of the superior constrictor muscle The anterior lateral face is formed by the parotid gland the ramus of the mandible and the internal pterygoid muscle The posterior wall of the pyramid is formed by the prevertebral fascia (Rogers 1956 Danforth 1963 Hora 1963 Levitt 1970) Besides the styloid and its muscles the space contains the internal carotid artery the internal jugular vein the ascending pharyngeal artery the hypoglossal the vagal the accessory the cervical sympathetic and the glossopharyngeal nerves

A parapharyngeal phlegmon or abscess may arise from a peritonsillar abscess penetrating the barrier of the superior constrictor muscle It may also arise from infected lymph nodes located in the parapharyngeal space draining the nose the nasopharynx and the tonsils A primary infection of the parotid gland may also occassionally give rise to parapharyngeal abscess (Weber 1950 Danforth 1963 Hora 1963 Levitt 1970)

In the cases here reported the diagnosis of parapharyngeal abscess was initially overlooked In both patients the most prominent symptom during the first days of the disease was a swelling of the parotid region and both patients were diagnosed as having an epidemic parotitis In both patients a peritonsillar swelling was observed later on At incision in the peritonsillar space no real abscess was found in either case Both patients presented neurologi symptoms In case 1 a Horner observed at an early stage of the

hypoglossal nerve paralysis devel ped later on Patient 2 developed symptoms from the cervi cal sympathetic nerves and all major cranial nerves running through the parapharyn eal space

The symptoms of our p t ts—swelling of the parotid gland region n s neurological symptoms and bulging of the lateral pha ryngeal wall medially ithout swelling or en largement of the t nsil— ree well with the clinical pictu of a raphary eal space abscess (B 19 Beck 1969)

Erosion of ... al car ... rom plicating a para ... ngeal ...

Infections of th ... raph ... ay at any time result ... ssels running through the ... sm (a dissecting ar ... rys ... ay ... ed with several ... bleedings ... om naso pharynx before ... final rupture ... avy fatal haemorr ... Salinger & ... man (1933) in a re ... the liter ture ... ring major pharyng ... ding follow ing ... ent kinds of deep ... fections inclu ... that of the paraphar ... eal space repor ... igh incidence of ... al haemorrhage ... ost mortem exami ations the interna ... rotid artery was found to be the most common ves sel involved while erosion of the external caro tid artery or the branches of this arter and of the internal jugular vein caused fatal bleeding to a lesser extent

DISCUSSION

The symptoms of erosion of the internal caro tid artery started several weeks after the pa tients in the present study fell ill and also several days after intense antibiotic treatment was started In case 1 swelling in the pharynx and in the parotid region had already subsided be fore bleeding was noticed Both patients devel oped a Horner syndrome and paralysis of the hypoglossal nerve It is evident from our cases and from previous reports (Metson & Van Nuys 1956 Eckel 1960) that the development of a Horner syndrome together with repeated minor bleedings from the nasopharynx are significant warnings of an internal carotid aneurysm, which must not be ignored The reason why the sympathetic nerve is generally affected may be the close anatomical rela tionship between the internal carotid artery and the sympathetic plexus running along the artery upwards through the carotid canal

In our cases the diagnosis of an internal carotid aneurysm was verified by *angiography* The great value of this investigation for dem onstrating the site of pharyngeal bleeding has previously been reported (Greven 1954 Hogarth 1959) In order to avoid an unneces sary ligation of the internal carotid artery an angiography should be carried out even though there are strongly suggestive symptoms of an internal carotid aneurysm

Killian (1951) concluded in a review of the literature of aneurysm of the head and neck vascular system that vascular grafting of the upper part of the extracranial internal carotid is impossible to perform He therefore recom mended ligation of the common carotid artery or of the internal carotid artery below the point of bleeding

Highly varying figures are reported of the incidence of cerebral complications after liga tion of the common or internal carotid artery (McCoy & Barsocchini 1968 Tindall & Odom 1969) The cerebral ischaemia following the ligation causes various neurological symptoms from mental confusion and hemiplegia to minor transient paresthesias in about 30% (Nishioka 1969) About 10% of the patients died from cerebral infarction after the ligation The complication rate increased with a e but serious ischaemic symptoms occasionally ap peared in children (Wisoff et al 1961 Nishi oka 1969) Neither of the patients reported by us developed any kind of cerebral complica tion after ligation of the internal carotid ar tery As regards cerebral complications it has been questioned whether ligation of the com mon carotid is a safer procedure than ligation of the internal carotid artery Tindall & Odom

(1969) in a review of the literature found no difference in the incidence of cerebral complications whether the common carotid or the internal carotid artery is ligated but other authors e g Nishioka (1969) have found the incidence to be lower after ligation of the common carotid artery A lower complication rate after ligation of the common carotid artery may be explained by a collateral circulation in the neck mainly from the inferior thyroid artery and a reverse flow in the superior thyroid and external carotid arteries to the ipsilateral internal carotid However the degree of this flow to the circle of Willis varies considerably In some patients there is instead a reversal of the flow in the internal carotid towards the external carotid (for review see Nishioka 1969 Tindall & Odom 1969) In view of the collateral circulation between the internal and external carotid—in one or the other direction—ligation of the common carotid artery does not guarantee safe control of a dissecting aneurysm of the extracranial part of the internal carotid artery In our opinion ligation of the internal carotid artery is therefore justified in patients with a dissecting aneurysm in its extracranial part

If the rupture of the dissecting aneurysm has already occurred a subsequent ligation of the internal carotid artery will not guarantee control of the bleeding as this may continue from the distal end of the rupture with reversal of the flow from the circle of Willis This is demonstrated in our first case If the internal carotid artery had been ligated at an earlier stage in that case thrombosis within the aneurysmal sac would probably have occurred and the rupture of the aneurysm with severe bleeding from the circle of Willis should have been avoided

In conclusion in patients with clinical symptoms of septic aneurysm of the extracranial part of the internal carotid artery—repeated minor bleedings from the nasopharynx and Horner s syndrome—an angiography should immediately be carried out to verify the diagnosis Subsequent ligation of the internal carotid artery should be performed as soon as possible despite the risk of cerebral ischaemia by this procedure

ZUSAMMENFASSUNG

Schwellungen am oberen Teil des Halses und in der Gegend der Parotis bei gleichzeitiger Anschwellung der lateralen Wand des Pharynx nach Pharynxinfektionen (Pharyngitis oder Tonsillitis) lassen auf einen parapharyngealen Abszess schliessen Bei einem derartigen Abszess konnen Komplikationen auftreten dadurch dass die in diesem Bereiche verlaufenden Nerven und Gefasse mitangegriffen werden In den untersuchten Fallen wurden neurologische Symptome am N glossopharyngeus N vagus N accessorius und N hypoglossus und an den zervikalen Sympathikusnerven sowie Erosionen der internen Arteria carotis beobachtet Bei der Erosion einer Arterie wird die Gefässwand entweder direkt perforiert oder es bildet sich im Allgemeinfalle vor der Ruptur der Gefasse ein Pseudoaneurysma Die fruhzeitige Diagnose eines Pseudoaneurysmas der internen Arteria carotis ist daher sehr wesentlich damit vor der oft todlich verlaufenden Ruptur eine Ligatur vorgenommen werden kann Es wird gezeigt dass wiederholte kleinere Blutungen von Nase und Hals und Horners Syndrom typische Symptome eines Pseudoaneurysmas der internen Arteria carotis sind Der Grund dafur dass der Sympathikusnerv angegriffen wird kann die anatomisch gegebene nahe Lage der internen Arteria carotis und des langs der Arterie durch den Karotiskanal aufwarts verlaufenden Sympathikusplexus sein

Die Bedeutung einer fruhen Diagnose eines Pseudoaneurysmas und der sofortigen Ligatur der internen Arteria carotis wird im besonderen erortert Zur Vermeidung von unnotigen Ligaturen wird vorgeschlagen zur Bestatigung der Diagnose eine Angiographie vorzunehmen

Das Auftreten von zerebralen Komplikationen nach Ligatur der internen Arteria carotis wird erortert

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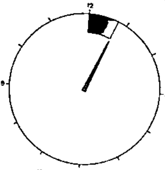
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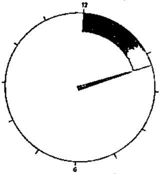
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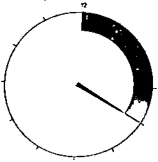
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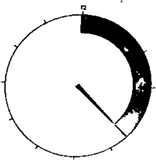


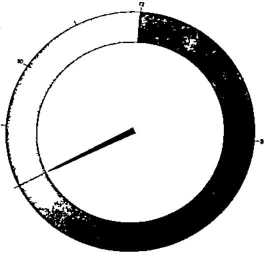
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